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Physiological and behavioural indicators of positive animal welfare in pigs

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LIST OF PUBLICATIONS RELATED TO THIS PhD THESIS:

1. Articles:

- Marcet Rius M, Cozzi A, Bienboire-Frosini C, Teruel E, Chabaud C, Monneret P, Leclercq J, Lafont-Lecuelle C and Pageat P 2018a. Providing straw to allow exploratory behaviour in a pig experimental system does not modify putative indicators of positive welfare: peripheral oxytocin and serotonin. Animal 12 (10), 2138-2146.

- Marcet Rius M, Cozzi A, Bienboire-Frosini C, Teruel E, Chabaud C, Monneret P, Leclercq J, Lafont-Lecuelle C and Pageat P 2018b. Selection of putative indicators of positive emotions triggered by object and social play in mini-pigs. Applied Animal Behaviour Science 202, 13-19.

- Marcet Rius M, Pageat P, Bienboire-Frosini C, Teruel E, Monneret P, Leclercq J, Lafont-Lecuelle C and Cozzi A 2018c. Tail and ear movements as possible indicators of emotions in pigs. Applied Animal Behaviour Science 205, 14-18.

- Marcet Rius M, Kalonji G, Cozzi A, Bienboire-Frosini C, Monneret P, Kowalczyk I, Teruel E, Codecasa E and Pageat P 2019a. Effects of straw provision, as environmental enrichment, on behavioural indicators of welfare and emotions in pigs reared in an experimental system. Livestock Science 221, 89-94.

- Marcet Rius M, Fàbrega E, Cozzi A, Bienboire-Frosini C, Descout E, Velarde A and Pageat P 2019b. Can environmental enrichment affect tail and ear movements in pigs, as potential indicators of emotions? Submitted in Animals, Special Issue of Environmental Enrichment of Pigs.

2. International congresses, Proceedings, Oral presentations, and Posters:

2.1. Oral presentations and Proceedings:

- Marcet Rius M, Cozzi A, Bienboire-Frosini C, Teruel E, Chabaud C, Monneret P, Leclercq J, Lafont-Lecuelle C and Pageat P. Crossing hormonal and behavioural measures to assess positive emotions in pigs. IVBM, Samorin, 2017. Prize for the best oral presentation by an ECAWBM-AWSEL Resident.

- Marcet Rius M, Pageat P, Bienboire-Frosini C, Teruel E, Monneret P, Leclercq J, Lafont-Lecuelle C and Cozzi A. Tail and ear movements as potential feasible indicators of emotions in pigs. ECBMAW, Berlin, 2018.

- Marcet Rius M and Codecasa E. Preliminary audit of minipigs involved in experimental procedures. Study Day ECBMAW-AWSEL, Berlin, 2018.

- Codecasa E and Marcet Rius M. The environmental enrichment, socialisation and training program for research animals: a team work. Study Day ECBMAW-AWSEL, Berlin, 2018.

2.2. Posters and Proceedings:

- Marcet Rius M, Pageat P, Ferraris C, Barthélemy H, Manteca X, Temple D, Mainau E and Cozzi A. The ergonomy of breeding: an innovative approach to improve welfare in pig production. ICPW, Copenhagen, 2015.

- Marcet Rius M, Cozzi A, Bienboire-Frosini C, Teruel E, Chabaud C, Monneret P, Leclercq J, Lafont-Lecuelle C and Pageat P. Playing or exploratory behaviour in pigs: neurohormonal patterns in mini-pigs and suggestions for welfare management. ISAE, Aarhus, 2017.

- Codecasa E, Marcet Rius M, Pageat P and Cozzi A. L'importance de l'enrichissement environnemental pour une chatterie et une porcherie d'expérimentation. AFSTAL, Reims, 2018.

- Fàbrega E, Vidal R, Marcet Rius M, Rodríguez A, Velarde A, Escribano D, Cerón J and Manteca X. Comparison of different enrichment materials to fulfil pig's behavioural needs and farmer's management system. ISAE, Charlottetown, 2018.

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RESUME en français

Le premier pas pour assurer une bonne qualité de vie des animaux de production pourrait être la possibilité d'évaluer correctement leur bien-être, en obtenant toute l'information possible sur leur « vrai » état de bien-être. Pour cela, il serait essentiel de ne pas seulement inclure la détection de quelques problèmes de bien-être, mais aussi la détection des états de bien-être positifs, comme l'émission d'émotions positives, qui, dans le long terme, pourraient être considérées comme du « bonheur ». En parallèle, être capable de mesurer l'apparition d'émotions négatives reste aussi essentiel, car, en définitive, il serait envisageable de considérer que le bien-être animal positif devrait inclure l'émission fréquente d'émotions positives associée à l'émission moins fréquente d'émotions négatives. Ainsi, la présence d'émotions positives et négatives devrait aussi être incluse dans l'évaluation du bien-être animal afin de tenir compte non pas seulement de la santé physique mais aussi de la santé mentale. Aujourd'hui, peu d'indicateurs existent mais seraient essentiels pour une meilleure compréhension de l'adaptation ou non à plusieurs pratiques effectuées dans les élevages et actuels systèmes de production. De plus, ce peu d'indicateurs ou

mesures déjà existants ne sont pas toujours faisables et objectifs à évaluer. En conséquence, il existe un besoin d'investiguer des nouveaux indicateurs de bien-être animal positif, et particulièrement, des réponses émotionnelles.

Ainsi, cette thèse a pour but principal d'examiner de potentiels indicateurs physiologiques et comportementaux du bien-être animal positif. Pour cela, nous avons mis en place cinq études, quatre avec portant sur des mini-porcs et une portant sur des porcs domestiques commerciaux. Les quatre études avec les mini-porcs ont été développés dans un cadre expérimental, tandis que la dernière le fut en conditions d'élevage. Une situation positive pour les animaux, en accord avec la littérature, a été créé dans toutes les études, et également, une situation contrôle, où plusieurs mesures ont été prises pour comparer les deux groupes.

La première étude intitulée *"Providing straw to allow exploratory behaviour in a pig experimental system does not modify putative indicators of positive welfare: peripheral oxytocin and serotonin",* présentée au Chapitre 1, a eu comme but d'investiguer l'effet potentiel de l'approvisionnement de la paille sur les niveaux de deux neuromodulateurs d'ocytocine et de sérotonine périphérique des mini-porcs. Comme l'approvisionnement en paille est largement reconnue comme étant bénéfique pour leur bien-être, il semblait intéressant de mesurer deux neuromodulateurs décrits comme étant liés à la gestion des émotions après l'approvisionnement, en comparant les résultats avec la situation contrôle, ainsi qu'en étudiant l'effet de l'approvi à court et long terme. Les résultats ont suggéré que la paille ne modifie pas ni la concentration de l'ocytocine ni celle de la sérotonine périphérique dans ce contexte.

La deuxième étude intitulée *"Selection of putative indicators of positive emotions triggered by object and social play in mini-pigs"*, présentée au Chapitre 2, était similaire à la première, mais dans ce cas le contexte positif consistait en la fourniture de jouets de taille moyenne, conçus initialement pour les chiens afin de permettre aux mini-porcs de jouer. Selon la littérature, le jeu chez les cochons est sensé leur évoquer des émotions positives. Nous avons obtenu des résultats intéressants en comparant les cochons qui jouaient avec ceux qui ne jouaient pas. Le résultat principal a montré que les animaux auxquels on avait fourni des jouets ont eu des niveaux plus stables d'ocytocine après les prises de sang après avoir joué que quand ils n'avaient pas de jouets. Cela a suggéré que ces cochons pourraient être dans un état émotionnel plus équilibré, due à la présence d'enrichissement environnemental et l'opportunité de jouer, en supportant mieux une situation stressante. Néanmoins, les cochons qui n'ont pas eu d'enrichissement ont montré une augmentation de l'ocytocine plasmatique à la suite des deux prises de sang, fait qui pourrait avoir activé le besoin de faire face à ("cope" en anglais) cette situation. Cela a suggéré que les mini-porcs qui pouvaient jouer étaient plus capables de faire face aux situations stressantes, fait qui serait très intéressant pour les actuelles pratiques d'élevage. Concernant la sérotonine périphérique, il n'y a pas eu de résultats significatifs, comme dans la première étude, en suggérant que cela n'était pas liée à la mise en disposition du matériel d'enrichissement. De plus, des corrélations positives ont été trouvées concernant les mesures physiologiques et comportementales, car depuis le début, nous avons observé des comportements intéressants pendant l'interaction avec l'enrichissement : une corrélation positive entre la fréquence de jeu avec l'objet et la durée du mouvement de queue, ainsi qu'entre la durée du jeu social et la durée du mouvement de la queue. Ces corrélations ont donné lieu à notre intérêt à poursuivre l'étude sur ces potentiels indicateurs comportementaux d'émotions positives chez le porc.

La troisième étude intitulée *"Tail and ear movements as possible indicators of emotions in pigs",* présentée dans le Chapitre 3, a montré que la durée du mouvement de queue était significativement plus haute quand les cochons jouaient que quand ils ne jouaient pas, tandis que la fréquence des mouvements d'oreilles était significativement plus basse. Comme le jeu est sensé produire des émotions positives, ces résultats suggèrent, en accord avec la littérature, qu'une durée élevée de mouvements de queue et une fréquence basse de mouvements d'oreilles sont liées aux émotions, en montrant un état de bien-être positif, et donc que ces mesures sont des indicateurs potentiels d'émotions chez le porc.

La quatrième étude, intitulée *"Effects of straw provision, as environmental enrichment, on behavioural indicators of welfare and emotions in pigs reared in an experimental system",* présentée au Chapitre 4, a montré que les indicateurs d'un bien-être pauvre (ou mal-être), tels un fort comportement agonistique un comportement de "displacement" et la fréquence de mouvements d'oreilles ont diminué avec la présence de la paille, en comparaison avec son absence. Néanmoins, les indicateurs potentiels d'émotions positives (durée et fréquence élevées de mouvements de queue) n'ont pas augmenté. Cela a suggéré que l'approvisionnement de la paille réduit l'émission d'émotions négatives et le mal-être, mais il semblerait qu'il n'entraîne pas l'émission d'émotions positives, en tout cas selon l'indicateur potentiel sélectionné dans cette étude (mouvement de queue).

La cinquième étude intitulée "*Can environmental enrichment affect tail and ear movements in pigs, as potential indicators of emotions ?",* présentée au Chapitre 5, a consisté à créer une situation positive pour les porcs d'engraissement en mettant en place du matériel d'enrichissement, analysant ensuite les potentiels indicateurs comportementaux de bien-être et mal-être: mouvements de queue et d'oreilles. Les résultats ont montré que la durée du mouvement de la queue était significativement plus élevée quand les porcs interagissaient plus avec l'enrichissement que quand ils le faisaient moins, pendant toute la période d'engraissement. Cela a suggéré qu'une durée de mouvement de queue élevée pourrait être un indicateur d'émotions avec un résultat positif (ou valence positive) chez le porc d'engraissement. Concernant la fréquence de mouvements d'oreilles, aucune différence significative n'a été obtenue entre l'interaction élevée ou faible avec l'enrichissement, contrairement aux résultats obtenus avec les mini-porcs dans des conditions contrôlées. Cela pourrait être due à la différence anatomique des oreilles entre les différentes races, qui pourrait influencer leur mobilité, en accord avec la littérature.

Ce travail a permis d'obtenir plusieurs résultats sur des potentiels indicateurs physiologiques et comportementaux de bien-être animal, qui pourraient être utilisés pour améliorer les actuelles méthodes d'évaluation de bien-être du porc. Concernant les indicateurs comportementaux, il a également apporté une caractéristique importante : la faisabilité des mesures. De plus, le travail a fourni une meilleure connaissance du vrai état des animaux et une meilleure compréhension de leurs émotions. Il a aussi contribué à avoir plus d'informations sur les différentes typologies de matériaux d'enrichissement, fait qui pourrait aider à résoudre l'actuelle difficulté à trouver le matériel le plus adéquat pour le porc, qui doit aussi être facile à gérer en élevage par les éleveurs ou techniciens. Finalement, ce travail est un exemple du lien entre différentes sciences, comme la physiologie, l'éthologie et le bien-être, entre autres, pour décrire scientifiquement l'état des animaux dans un contexte précis.

MOTS CLES

Bien-être animal ; Bien-être positif ; Emotions ; Ocytocine périphérique ; Sérotonine périphérique ; Mouvement de queue ; Mouvement d’oreilles ; Matériel d’enrichissement ; Méthode d’évaluation de bien-être du porc ; Production du porc.

ABSTRACT

The first step for ensuring a good quality of life for farm animals may be the ability to assess their welfare correctly by obtaining all of the information about the real state of their welfare. To do this, not only is it essential to include the detection of welfare problems but it is also essential to detect positive welfare states, such as the emission of positive emotions, which in a long-term situation might be considered to be “happiness”. The ability to measure the appearance of negative emotionsis important, and positive animal welfare should also include frequent emissions of positive emotions and less frequent emissions of negative ones. Therefore, the presence of positive and negative emotions should be included in animal welfare assessments, and not only their physical health but also their mental health should be considered. Currently, few indicators of emotions exist, and such indicators are essential to better understand the adaptation or not of several husbandry practices in current production systems. Furthermore, the few existing indicators or measures are not always feasible and objective to evaluate. Therefore, research identifying new indicators of positive welfare, more specifically, indicators that reflect emotional responses, is needed.

Thus, the present work mainly aimed at investigating potential physiological and behavioural measures of positive animal welfare. For this investigation, we conducted five studies: four with mini-pigs and one with domestic commercial pigs. The four studies with mini-pigs were performed in an experimental setting, whereas the last study was performed under farm conditions. In each study, a positive situation was created for the animals according to the literature as well as a control situation, and several measurements were taken to compare both groups.

The first study, entitled "*Providing straw to allow exploratory behaviour in a pig experimental system does not modify putative indicators of positive welfare: peripheral oxytocin and serotonin"* and presented in Chapter 1, aimed at investigating the potential effects of straw provision on peripheral oxytocin and serotonin. As straw provision in pigs is widely presumed to be highly beneficial for their welfare, measuring peripheral oxytocin and serotonin after straw provision and comparing the measurements to those made in a control situation, as well as analysing the short- and long-term effects of oxytocin and serotonin, was of interest. The results suggested that straw provision to pigs does not modify the oxytocin or serotonin concentrations in a controlled system.

The second study, entitled *"Selection of putative indicators of positive emotions triggered by object and social play in mini-pigs"* and presented in Chapter 2, was very similar to the first one, but this time, the positive context consisted of providing medium-sized dog toys to trigger play behaviour. According to the literature, playing is supposed to produce the emission of positive emotions in pigs. A comparison of pigs that played with control pigs provided us with many interesting results. The main result was that for the animals that played, when toys were provided to them, they showed more stable levels of oxytocin after playing and after blood sampling. This finding suggests that those pigs may already be in a balanced state of welfare due to the presence of environmental enrichment and opportunities to play, thereby providing better support during a stressful event. However, the pigs that did not receive the enrichment material, showed an increase in plasma oxytocin following two blood sampling events that could have activated a need to cope. These results suggest that the pigs that were allowed to play were more capable of coping with stressful situations, which would be of use in current husbandry practices. Concerning peripheral serotonin, no significant results were found, as in the first study, suggesting that serotonin was not linked to the provision of enrichment material in pigs. In addition, some positive correlations were found in the physiological and behavioural measures. For example, we observed some interesting behaviours in pigs during the interaction with the enrichment: a positive correlation with object play frequency and tail movement duration, as well as between social play duration and tail movement duration. These correlations gave rise to our interest on this behaviour as a potential indicator of positive emotions in pigs.

The third study, entitled *"Tail and ear movements as possible indicators of emotions in pigs"* and presented in Chapter 3, showed that tail movement duration was significantly higher when pigs played than when they did not, whereas ear movement frequency was significantly lower. As play behaviour is supposed to trigger positive emotions, these results suggest, according to the literature, that a high tail movement duration and low ear movement frequency are linked to emotions, showing a positive state of welfare and thus representing potential indicators of emotions in pigs.

The fourth study, entitled *"Effects of straw provision, as environmental enrichment, on behavioural indicators of welfare and emotions in pigs reared in an experimental system"* and presented in Chapter 4, showed that indicators of poor welfare (agonistic behaviour, displacement behaviours and ear movement frequency) decreased with the presence of straw compared to the absence of it. Nevertheless, the potential indicators of positive emotions (high tail movement duration and frequency) did not increase. These results suggest that straw provision reduces negative emotions and negative welfare in pigs, but does not seem to produce positive emotions, at least as shown by our selected potential indicator (tail movements).

The fifth study, entitled “*Can environmental enrichment affect tail and ear movements in pigs, as potential indicators of emotions?”* and presented in Chapter 5, consisted of creating a positive situation for fattening pigs by providing enrichment materials and analysing the potential behavioural indicators of welfare: tail and ear movements. The results showed that tail movement duration was significantly higher when the pigs interacted more with the enrichment than when they did less during all of the fattening period. This result suggested that a high tail movement duration could be an indicator of positive emotions in fattening pigs. Therefore, tail movement can apparently be used to indicate positive emotions and positive animal welfare not only in mini-pigs but also in commercial pigs. Concerning ear movement frequency, no significant differences were found between the high interaction or low interaction with the enrichment, contrary to the results obtained with mini-pigs under controlled conditions. This difference may have been because of the anatomical differences between the ears of the breeds, which according to the literature, may reflect a difference in ear mobility, among other possibilities.

This work provides many interesting results about the potential physiological and behavioural indicators of welfare that could be used to improve current welfare assessments of pigs. The present behavioural indicators are important because they provide measures that are feasible. Furthermore, this work provides further knowledge of the real state of animals and a better understanding of their emotions. This work also provides information about different types of enrichment materials, which could help stockpersons and others to manage pigs find adequate material for enrichment. Finally, this work is an example of the way many different sciences, such as physiology, ethology and welfare, among others, can be linked to scientifically describe the state of animals in a specific context.

KEYWORDS

Animal welfare; Positive welfare; Emotions; Peripheral oxytocin; Peripheral serotonin; Tail movement; Ear movement; Enrichment material; Pig welfare assessment; Pig production.

INDEX

GENERAL INTRODUCTION

1. Ethology of pigs

1.1. Introduction:

Ethology could be defined as the science that studies the spontaneous behaviour of animals under natural conditions, how the behaviour is produced (ontogeny) and its function and evolution (phylogeny) (Manteca 2009).

Behaviours are influenced by genetic factors (by natural selection) and by the environment. Importantly, when we modify their environments, not all animals modify their behaviour, as their behaviour is influenced by their genetics (Manteca 2009).

Domestication has modified some natural behaviours in animals, as selection occurs toward the most adaptable animals. Nevertheless, many natural behaviours are maintained after domestication, with animals retaining their motivation to perform a behaviour, even if they have never had the opportunity to perform it under kept conditions, e.g., nesting behaviour by sows.

Knowing the ethology of domestic species is important because their natural behaviours allow us to understand their needs and the difficulty they have when adapting to the conditions provided by humans (Stolba and Wood-Gush 1984; Heffner and Heffner 1990). Therefore, to provide farm animals with a good quality of life, the first step should be to measure the real state of welfare in this context, to be able to suggest some improvements to solve the potential problems.

In this chapter, we are going to describe some natural behaviours of pigs, especially those behaviours that could be more affected in intensive pig production systems.

We summarize the ethology of pigs in two parts: their perception and interaction with the environment and their social interactions.

1.2. History and domestication of pigs:

Domestication is a process of adaptation of a population of animals or plants to conditions created by humans. This process includes two types of changes: genetic changes, which are transmitted from the parents to their offspring, and environmentally induced changes, which are repeated in every generation. This means that domesticated animals survive and reproduce better under the conditions provided by humans than their wild ancestors (Manteca 2009).

An understanding of the domestication process is important because the behaviour of domestic animals is the result of two elements: the behaviour of the wild ancestors—in this case, the wild boar—and the behavioural changes caused by the domestication. In addition, understanding that the ethograms of all domestic species are almost identical to those of their wild ancestors is important because this fact explains the origin of many welfare problems in intensive production systems (Manteca 2009).

Different theories exist about the domestication of pigs. Two different domestications are believed to have occurred in wild boars (*Sus scrofa*); these were carried out in two different places, Anatolia and China, during the period between the

11.000 and 7.000 years before Christ (Giuffra et al. 2000; Lega et al. 2016). Other studies, however, have suggested that other additional independent domestications of wild boar took place in India, in the Southeast of Asia and on the coast of Taiwan (Larson et al. 2010). Some authors have suggested that swine arrived in Europe around the 5.000 BC, when people from central Asia moved to Europe.

The wild boar (*Sus scrofa*) was an important prey to hunter-gatherers across wide areas of Eurasia until the early Holocene (Benecke 2017; Pushkina and Raia 2008), and currently, it is one of the most widespread wild mammals. Similarly, its domestic form, the pig, rapidly gained economic importance (Lega et al. 2016).

1.3. Perception and interaction with the environment:

We consider knowledge about the perception of pigs to their environment as an important aim to understand most pig behaviours.

1.3.1. The five senses:

The five senses are sight, hearing, olfaction and chemical communication, touch and taste.

Pigs are prey species, and that is why they have a large angle of view, in order to see their predators. More precisely, their angle of view is 310°, and the angle of binocular vision is around 50° (Adamczyk et al. 2015). This allows them to observe very well their surroundings, but it reduces its capacity to determine the distance of objects because of the monocular vision on the sides (Manteca 2009). Many studies showed that they are able to distinguish colours (Eguchi et al. 1997; Tanida et al. 1991; Houpt 2005) and that they have some colour preferences (Klocek et al. 2016). For ex: it seems that they are not able to distinguish red from green, but they are able to distinguish blue from green (Tanida et al. 1991).

Because their hearing is much more sensitive, pigs perceive many noises that humans are not able to hear. They are especially more sensitive to acute noises.

The hearing of pigs range from 42Hz to 40,5 kHz with a region of best sensitivity from 250 Hz to 16kHz (Heffner and Heffner 1990). Pigs' hearing range is similar to that of humans, but with a shift toward ultrasound (Talling et al. 1996). Pigs show an aversion to sudden loud noise, especially for a frequency of 8 kHz and for an intensity of 97 dB (Talling et al. 1996).

Concerning the olfaction and chemical communication, this sense is extremely important in pigs, and it is much more developed than in humans. They use it for intraspecific and interspecific communication. This sense is involved in social communication, reproduction, maternal behaviour, identification between animals and feeding, among others (Signoret and Mauleon 1962; McGlone 1990; Sommerville and Broom 1998; Pageat 2000).

Pigs have a good sense of touch, and it is possible to observe how they scratch themselves, how they are bothered by flies and how they like to be in contact with other mates. Touch is detected through nerve endings in the skin and subcutaneous tissue, the snout is the primary organ of touch for the pig. However, through the foot pad the pig will identify changes in flooring texture and will hesitate at surfaces for which it is not accustomed. Touch provides a way to communicate, and it is used in rooting behaviour, in exploratory behaviour and in sexual behaviour, among others (Manteca 2009).

Finally, concerning the sense of taste, pigs have taste papillae on the tongue, epiglottis and soft palate epithelia (Montavon and Lindstrand 1991). Each papillus contains high numbers of taste buds (Roura and Tedó 2009). Taste buds are composed of at least four different types of cells: taste-type cell I, II and III, and one basal-type cell, the progenitor of the other three. These cells allow the pigs to distinguish sweet, umami, bitter, sour (acid) and salty (Kittawornrat and Zimmerman 2010). Many studies have reported a preference for a sweet taste (Jacela et al. 2010) and, for this reason, sweet flavoring agents have been used for creating interest in solid food in weanling pigs (Forbes 1995).

1.3.2. Behavioural needs: rooting and nesting behaviours:

Behavioural needs are identified in each species when a particular behaviour is restricted, and as a consequence, negative consequences appear: a stress response, a redirected behaviour or a stereotypy. Animals are very motivated to perform these behaviours, and this motivation would only disappear after performing the behaviour (Jensen and Toates 1993).

These behaviours have allowed species survival, development and reproduction (Studnitz et al. 2007). Even after the domestication process, pigs have conserved the same behavioural needs: rooting behaviour and nesting behaviour. The difference between them is that the motivation to root is permanent, but the motivation to perform nesting behaviour only occurs before farrowing.

1.3.2.1. Rooting and exploratory behaviour:

Pigs are omnivorous animals, their natural sources of food are found sporadically within a large home range, and under natural conditions, pigs spend a large part of their active time searching for food by exploring their surroundings (Studnitz et al. 2007). For instance, Stolba and Wood-Gush (1989) found that domestic pigs living in a semi-natural environment spent 52% of the daylight period foraging (rooting and grazing) and another 23% in locomotion and direct investigation of environmental features. Although the pig has been domesticated and fed by humans for several generations, it continues to have the same motivation to explore and root under prevailing conditions (Studnitz et al. 2007). Pigs explore their surroundings by rooting, sniffing, biting and chewing various food items as well as indigestible items. In this way they become familiar with their environment and the various resources within it. The above-mentioned behavioural elements may also occur in other contexts than exploration; for instance, chewing may also be viewed as consummatory behaviour.

Under intensive pig production conditions, even when mandated by law, most farms do not provide manipulable material to allow the pigs to perform rooting behaviour. In addition, the close confinement reduces the possibility of pigs exploring and investigating their environment (Broom and Fraser 2015), which increases the probability that animals will suffer many animal welfare problems, such as tail biting, which has a multifactorial aetiology but is certainly influenced by the lack of substrate.

1.3.2.2. Nesting behaviour:

Under natural conditions, one day before farrowing, a sow will leave her sow group and start to build a nest, with the aim of creating a safe and comfortable place for her and her offspring (Burne et al. 2000). Specifically, nesting behaviour consists of leaving the sow group, searching for material for the nest, bringing the material to a quiet area (generally in a forest), making a hole, building the nest, and staying there until approximately two days after the farrowing. Because the sow is much larger than the piglets, she can crush them with some movement, which occurs frequently; thus, nesting behaviour and the corresponding resting behaviour in the nest reduces the probability of crushing. Another reason for this behaviour is that sows do not lick their offspring, so the offspring should be protected in some hot and quiet place so they can dry safely. Furthermore, new-born piglets do not have brown adipose tissue (BAT), which has a thermoregulatory function, so they are more vulnerable during the first hours of life (Manteca et al. 2014).

Under intensive pig production conditions, sows are not allowed to perform nesting behaviour, which increases stress and, consequently, makes the farrowing more difficult. If farrowing takes longer, the piglets will be more hypothermic and starved, which increases the probability of piglets being crushed, resulting in higher mortality.

1.3.3. Elimination behaviour:

Pigs are very clean animals. Under natural conditions, they eliminate in areas that are far away from resting and feeding areas. This behaviour starts at one week of age. Their home range is approximately 100-500 ha, allowing room for these areas to be separate.

The lack of space and the high density in intensive pig production systems limit the performance of this behaviour, as pigs are not able to divide their pens into three areas (feeding, resting and elimination areas) (Muñoz Luna 2006). Consequently, they suffer discomfort, frustration and stress, which would affect their welfare and performance. The dirtiness of the pigs, which is due to the lack of possibility of dividing the pens into the three areas, is considered an indicator of poor welfare because of the dirtiness of itself and because of the potential increase of stress on the animals.

1.4. Social behaviour and social interactions:

Social behaviour is composed of all the behaviours between two or more individuals, including the aggression, the space, the reproduction, the maternal behaviour and the social organisation (Keeling and Gonyou 2001).

1.4.1. Composition and structure of the group:

The most typical group of pigs is composed of sows and their most recent offspring living in their territory or home range. These sows are of the same family, with most being sisters or half-sisters. External sows are not usually accepted in the group. The number of individuals in each group depends on the quantity of resources, with groups being larger when more resources are available.

At six or seven months of age, young males separate from the group of sows and offspring, creating small groups of two or three males until the reproduction period, when the young males integrate with a group of sows. Once this period is finished, the males will again form a group, where they will remain until they reach adulthood at three years of age; at that time, they become solitary except during the reproduction period.

In an intensive pig production system, the kinship of sows was not considered in the past, a fact that affected the aggressivity and the non-acceptance of some members of the group.

1.4.2. Hierarchy:

Pigs organise their groups hierarchically. They are more dominant or more subordinate depending on their disposition.

We can divide the type of individual into three categories: the ones who are very active and very implicated in social interactions; the ones who are active and implicated in social interactions, but with less success; and the ones more inactive and show less interest in social interactions. These three categories compose the scale of dominance in a group, starting with the most dominant and finishing with the most subordinate. The animals in the middle will be subordinate to some individuals and dominant to others.

The reason pigs are organised by hierarchies is to allow individuals to avoid fighting every time they want to do something at the same moment and place as another pig. Once a hierarchy is established, fighting is unusual, as the individual to have the preference is usually clear.

Hierarchy is established in new-born piglets at approximately two days of age (Houpt 2005). They are able to establish hierarchy faster when they are younger. That is why the mixing of animals in intensive pig production systems is not recommended at all, and if it "must be done", it should be done as soon as possible to reduce fighting between animals. Nevertheless, the continuous mixing of fattening pigs is very common in the production system, as well as the mixing of sows, which contributes to continuous fighting as individuals in the new groups attempt to establish hierarchies, producing significant lesions, miscarriages and even mortality.

1.4.3. Agonistic behaviour:

Agonistic behaviour is a continuum of behaviours expressed in conflict situations and includes offence, defence and submissive or escape components. The behaviours involved may include contact, such as biting and pushing, or non-contact, such as threats in the form of body postures and gestures (Petherick and Blackshaw 1987).

Agonistic behaviour is important for the establishment of a dominance hierarchy among new group members (Meese and Ewbank 1973).

It involves situation conflicts that are resolved by fighting, threatening or escaping. This type of behaviour occurs more frequently in males than in females, due to sexual hormones, and it occurs at all ages.

Under natural conditions, this behaviour is not excessive, as unknown pigs are not enclosed in a small place where they sometimes need to compete for resources. However, this behaviour does occur under intensive conditions.

Under commercial conditions, agonistic behaviour is caused mainly by the mixing of pigs, some methods of feeding, the lack of space, the lack of manipulable material, the lack of hiding places and the size of the groups, among others (Petherick and Blackshaw 1987).

Post-weaning aggression after the mixing of piglets of different mothers presents a significant cost to animal welfare and economic efficiency (Jensen and Wood-Gush, 1984) due to stress and injury. The literature shows that post-weaning aggression is significantly higher in pigs reared under poor conditions compared to those reared under enriched conditions (De Jonge et al. 1996; Olsson et al. 1999). Pre-weaning housing systems may not only affect the aggression that occurs immediately following the mixing of litters at weaning but also affect the agonistic behaviour that occurs in the long term (Chaloupková et al. 2006).

1.4.4. Allelomimetic behaviour:

Allelomimetic behaviour consists of the imitation of the behaviours performed by other members of the group. Its function is to maintain the group and provide security; for instance, if one pig sees a danger, it would be scared and react to save itself, and the other pigs would immediately do the same.

The inclusion in this study of observations on emotional contagion between pigs, which essentially would be a sort of allelomimetic behaviour, would be interesting. Some authors have shown that when a pig experiences positive or negative emotions, these feelings can be transmitted to other pigs that, in turn, could experience the same emotion and therefore perform the same behaviour (Reimert et al. 2013 and 2017).

Such transmissions could have important implications for the welfare of group-housed pigs in intensive pig production, suggesting a field of research that needs to be explored.

1.4.5. Maternal behaviour:

Maternal behaviour is the care a mother provides to her offspring. Maternal behaviour can be more or less developed and effective depending on the following:

- whether the mother was a gilt before farrowing (primiparous) or a sow (multiparous) because the sows with more experience are less stressed and, consequently, are usually better mothers;
- the breed, as some breeds have more developed maternal behaviours than others;
- genetic variations within the same breed, such as when sows are selected for a character that, at the same time, decreases maternal behaviour;
- stress, which can influence maternal behaviour considerably; and
- the possibility or not to perform nesting behaviour, which increases stress.

The most common consequences of poor maternal behaviour in intensive pig production systems are cannibalism (Broom and Fraser 2015), the rejection of the piglets and the lack of reaction to the screams (distress calls) of the piglets when they are being crushed (a sow showing correct maternal behaviour will stand up immediately to avoid crushing the piglet) (Manteca et al. 2014).

1.5. Conclusion and transition:

Following this description of some of the natural behaviours of pigs, we are going to describe the living conditions of domestic pigs, particularly in intensive pig production systems, which account for more than the 90% of the actual European production.

2. Pig production system

2.1. Introduction:

In this chapter, only general practices of intensive production are described, and these occur in more than the 90% of production systems. Thus, alternative systems, which, when correctly implemented, are generally more respectful with the ethology and welfare of the pigs, are excluded from this study.

2.2. Intensive pig production system:

2.2.1. General aspects:

Understanding the general practices and management of the intensive husbandry systems and the reasons for farmers and technicians organising production in that way is important. General aspects of the intensive pig production system are summarized here for a better understanding of the actual situation and the aim of this thesis.

Traditionally, the first artificial insemination or coverage occurs at seven or eight months of age in the sow and at eight, nine or ten months of age in the boar. The sows are held in individual crates at the moment of insemination.

The gestation lasts approximately 114 days (from 113 to 116), i.e., three months, three weeks and three days. Sows are moved from individual crates to a group housing system at four weeks of gestation. They are housed in groups until the week before the farrowing, when they are moved to the farrowing crates.

Sows are housed in the farrowing crates until three or four weeks after farrowing, when the weaning is undertaken. At that time, the piglets are moved to a group housing system for the post-weaning period and afterwards moved to other facilities for the fattening period, also in group housing. Some female piglets will be used for "replacement" of older sows on the farm. After weaning, the sows return to the individual crates for the next insemination, which occurs approximately one week after weaning.

2.2.2. Sows:

Sows can be in different periods of production:

- Insemination or coverage and the first period of gestation – individual crates
- Gestation – group housing pens
- Maternity and lactation – farrowing crates

The first reproductive cycle usually is at six months of age, and the first insemination is at seven or eight months of age, with gilts having a minimum weight of 135 kg. The sows are seasonally polyoestrous species, meaning that they are in oestrus throughout the year. Once they begin to be regular in their cycles, they are going to be in oestrus every 18-24 days. They usually farrow 2.2 times per year.

After the piglets are weaned, the sows tend to go into heat at three, four or five days. They are inseminated at seven, eight or nine days after the weaning.

During this period (4 weeks after the farrowing), the sows are housed in individual crates, where they can only stand up, sit down and do one or two steps depending on their size. Importantly, before the directive of the 2001/88/EC, the sows were continually housed in these crates and in farrowing crates.

Figure 1: Sows housed in individual crates. Intensive pig farm located in Zaragoza, Spain, 2014. Picture taken by M. Marcet Rius.

The gestation of the sow lasts three months, three weeks and three days (114 days). The most select breeds, which are bred in intensive production, typically have approximately twelve to fourteen piglets per service and 2.2 services per year. That means each sow produces approximately 26-28 piglets per year.

Currently, regarding the housing, sows are in cages until four weeks of gestation, when they are moved to the group pens. They will be there until the week before the farrowing, when they are moved to farrowing crates. Since 2013, members of the European Union are obliged to house pregnant sows in groups during this period, thanks to directive 2001/88/EC.

This group housing system is designed to satisfy the needs of pregnant sows and increase the level of animal welfare. Some separators are recommended to allow the sows to protect themselves if they are being attacked by other sows. If there are some very aggressive sows and sick or injured sows, those can be housed temporarily in individual enclosures.

The group housing system could be static or dynamic, depending of the management. The static groups are those that are always composed by the same animals. They are composed by sows from the same batch of insemination, so all the sows are in the same physiological state of pregnancy. The dynamic groups are those where different individuals are introduced progressively, with sows being in different states of gestation and sometimes, unknown to one another.

The ideal situation would be a static group with sows that are sisters and half-sisters, to avoid excessive fighting during the establishment of hierarchy.

Feeding can be administered in many different ways. Some methods prevent competition between animals, such as some electronic systems, and other methods do not prevent competition. A correct feeding administration is essential to guarantee a minimum state of welfare, which reduces excessive fighting between sows.

Figure 2: Group-housing system of pregnant sows. Intensive pig farm located in Zaragoza, Spain, 2014. Picture taken by M. Marcet Rius.

One week before the farrowing, sows are moved to the farrowing crates. Farrowing is a stressful time for the sow and the piglets, so breeders need to be prepared to avoid problems.

Farrowing crates are designed to reduce the crushing of the piglets, even if it is not eliminated in general. The piglets can move freely, but not the sow. The floor is usually slatted, and it has a heated area for the piglets, where they sleep.

Figure 3: Sows and piglets in farrowing crates. Intensive pig farm located in Zaragoza, Spain, 2014. Picture taken by M. Marcet Rius.

2.2.3. Piglets:

Piglets remain with their mother for three or four weeks, living in maternity cages. During the first days, some painful and stressful procedures are applied:

- At one or two days of age, even if not recommended, tail docking and teeth clipping are performed. The traditional justification still used is that, tail docking avoids tail biting, even though proven not true; for the teeth clipping, the justification is that it prevents lesions on the sow's teats, as well as the lesions on piglets and future pigs from fighting, even if other measures should be provided to avoid it. These practices do not prevent the mentioned problems, they cause acute and chronic pain, and they are often performed without anaesthesia or analgesia. Unfortunately, most farms continue these practices, which are still not legally prohibited.
- Piglets are identified with a tag.

- Iron and vitamins are administered.
- Piglets are castrated on many farms and in many countries despite the efforts of the European Commission and animal welfare organisations, which have been fighting to prohibit the procedure due to the brutal techniques used and the acute and chronic pain caused to the piglets. The justification given is that it prevents the boar taint and excessive fighting in the last period of fattening, that occurs when pigs are intact. Many alternatives have been proposed to substitute for castration, such as breeding intact males with some slight modifications to management or possibly immuno-castration. Some countries have already adopted one of these techniques, as the European Commission stated many years ago that castration would be forbidden in 2018 (but it is still not done), but some of them continue to castrate with no modification. The last directive (2008/120/EC) ordered that piglets castrated after one week of age must receive anaesthesia and analgesia. If done before one week of age, that is not necessary. Many studies have shown that immature animals, such as piglets of less than one week of age, can suffer and perceive pain in exactly the same way as an adult individual, or even more so. Unfortunately, the law advances much more slowly than the science, and we still have the same directive allowing the castration of the animals without anaesthesia or analgesia.

After being weaned, piglets are moved to other pens where different offspring from different mothers are mixed. The weaning is abrupt, with no progressive, so animals are separated from their mothers at three or four weeks of age (very soon compared to their natural behaviour), when they will transition in the first fattening period. This early separation can have consequences for their welfare, such as an excessive sucking behaviour of other piglets (belly-nosing behaviour) (Broom and Fraser 2015).

2.2.4. Fattening pigs:

The initial fattening period can be referred to as the “transition period” or “post-weaning”. This phase consists of the period between weaning and two or two and a half months of age, so it lasts approximately one or one and a half months, until the time the weaners weigh 20-25 kg, when they can be considered rearing or fattening pigs.

After being weaning, the piglets are moved to a group housing system with a variable number of animals, but these groups are typically large, with approximately 30 animals. The offspring of many different mothers are mixed in those pens, and they are separated and divided progressively in different pens according to their weights, to create homogeneous groups. In general, this technique produces a high number of mixing situations, with corresponding fighting between animals to establish new hierarchies with each change, creating an important welfare problem.

The fattening period finishes when the pigs weigh 100-110 kg, at five and a half or six months of age. At that moment, the pigs are sent to the slaughterhouse. The age of the slaughter also depends on the sex of the animals; if they are intact males, they will go before the castrated males or the females, to avoid excessive fighting and mounting behaviour and, especially, to prevent the boar taint.

During the fattening period, groups used to comprise 10-12 animals, but in some farms, groups can be much bigger (approximately 30 animals). Farms house either entire males, castrated males or females. This housing is better because it better meets the needs of each sex and produces more homogeneous groups, which is important for the production of meat.

The floor is usually a slatted floor. The temperature needs to be approximately 20 °C (higher at the beginning of the fattening period and lower at the end). Feeding can be provided in many different ways but is almost always provided ad libitum.

Figure 4: Fattening pigs. Farm located in Catalonia, Spain, 2017. Picture taken by M. Marcet Rius.

2.2.5. Animal Welfare considerations:

This explanation of the functioning of intensive pig production systems, considered in concert with some of the natural behaviours of pigs, indicate that many animal welfare problems will be evident in these systems.

Importantly, much individual variability exists in the ability of an animal to cope with the different situations. This variability means that, in every difficult situation or problem that we expose, we will find some animals that are very affected and others that are more tolerant and cope better in that difficult situation, even if the conditions are identical or very similar. For example, when sows were continuously housed in individual crates, some but not all showed stereotypies, such as bar biting. This behaviour is due to the individual personality or susceptibility and individual capacity to cope with the environmental or housing issues.

In this chapter, the most common animal welfare problems are going to be described: specifically, problems in intensive pig production systems, and more precisely, problems that occur during the production phase (not in transport or in the slaughterhouse).

Some of these animal welfare issues are as follows:

- Frustration and stress occurring because it is impossible for pigs to perform exploratory and rooting behaviour
- High density, insufficient space
- Aggressiveness between pigs and excessive fighting after mixing
- Occurrence of stereotypies and redirected behaviours
- Stress and fear from an inadequate handling or a negative human-animal relationship

- High culling rate of sows
- Health problems of sows and pigs
- Neonatal mortality
- Painful procedures in piglets
- Impossibility of the sow to move in farrowing crates and individual crates

An explanation is provided of each problem being exposed, as well as its relation to or treatment by existing law.

2.2.5.1. Frustration and stress incurred because it is impossible for the pigs to perform exploratory and rooting behaviour:

In nature, pigs perform exploratory and rooting behaviour (search, selection and consumption of food and water) for approximately seven hours per day, whereas in intensive production, they usually do not have an opportunity to root, as the floor is concrete/slatted, and no manipulable material is provided.

The availability of a rooting substrate may profoundly affect the behaviour and welfare of pigs (Bolhuis et al. 2006). Consequently, the lack of rooting substrate, among many other welfare problems, can produce some redirected behaviours, such as biting on different parts of their pen mates, especially the tails, which is very well known as tail biting and is a big problem when outbreaks occur.

Tail biting is an important animal welfare issue, and also produces high mortality when it appears. Therefore, the provision of adequate environmental enrichment that allows the pigs to perform exploratory, rooting and chewing behaviours is very important. The best enrichment material identified to date, which allows the performance of these behaviours and consistently reduces many animal welfare issues, such as redirected behaviours and stereotypies, is straw (van de Weerd and Day 2009). However, currently, facilities do allow for the provision of straw in a practical and economical manner, so it is not feasible.

Directive 120/2008/EC obliges these farms to provide manipulable material, but unfortunately, this directive is not followed by most farms. Therefore, research to find some feasible manipulable material is being encouraged in Europe (European Food Safety Authority 2014).

2.2.5.2. High density, insufficient space:

In general, pigs are not allowed to divide their pens into the three desired areas (resting, eliminating and feeding areas), as the space is insufficient.

Under natural conditions, they eliminate far away from the resting and feeding zones. Moreover, they have a home range between 100 and 500 ha, where they have the possibility of walking, exploring, feeding and resting, activities that are very difficult under intensive conditions. This frustration caused by the impossibility of performing almost any of these behaviours and chronic stress result in immunodepression and an increase in susceptibility to many diseases.

Directive 91/630/EC obliges farmers to provide a minimum space per pig with regard to the pig's weight, and that obligation has been repeated in the following directives. Nevertheless, this space is really very small and does not allow the animals to perform their natural behaviours.

2.2.5.3. Aggressiveness between pigs and excessive fighting after mixing:

Pigs are mixed frequently to obtain homogeneous groups of weights, and each time, they need to re-establish their hierarchies. Under natural conditions, this high quantity of aggressions does not exist, as animals are not enclosed in a reduced space and continuously mixed with unknown individuals, where they may compete for resources.

This continuous mixing of animals is antithetical to animal welfare as pig ethology is not considered at all. For example, in the group housing system of sows, when different individuals are introduced into the group every week, excessive fighting occurs for the establishment of the hierarchy, which also increases the number of miscarriages and influences performance. The reason for fighting is not that the sows are very aggressive and should be housed alone (which has been an important discussion for many years); instead, the reason for the fighting is that the management and organisation of the groups are not correct (Verdon et al. 2015). Additionally, after weaning, piglets are mixed with other litters, and unfortunately, it is not the only time when pigs are going to be mixed. A common practice of farmers consists of mixing animals throughout the fattening period to create homogeneous groups of weights.

Of course, the feeding system can encourage apparent aggressiveness; when systems are adopted that force animals to compete for food, aggressions increase substantially.

Directive 2001/93/EC, as well as 120/2008/EC, established that when mixing between pigs has to be done, it should be done as soon as possible and is allowable until one week after weaning. These directives also established that the most aggressive animals and those experiencing the most aggression could be housed in different pens and that when excessive fighting is occurring, some measures should be organised to reduce it, such as the provision of a high quantity of straw.

Importantly, the management of each farm is different, and even when a method exists that is common and frequently applied, not all farmers engage in the practice.

2.2.5.4. Occurrence of stereotypies and redirected behaviours:

Stereotypies and redirected behaviours may indicate an animal is attempting to cope with the environment, but this adaptation comes at a cost to the animal. When an animal is facing a difficult situation, a stress response is activated. Although this response can be very useful for the animal, when the stress is intense or prolonged, the response can have very negative effects, such as a decrease in growth, inhibition of the reproductive function and depression of the immunity system.

Stereotypies and redirected behaviours are some of the abnormal behaviours induced by an inadequate environment.

Stereotypies are defined as repetitive behaviours, invariable and without apparent function, which appear in aversive environments for the animal and frequently have aversive effects for the health and the productivity (Broom 1983). Therefore, these behaviours are indicators of poor welfare.

The most common stereotypies in sows and pigs are bar biting, continuous biting and continuous manipulation of the drinker.

In sows, the stereotypies are mainly produced by hunger due to restricted feeding. Some studies have shown that food restriction for sows is not justified because, if the food is increased (over what it is generally recommended), the stereotypies are reduced and the growth of future piglets will be higher, with lower conversion rate in the future (Manteca and Gasa 2005). Increased feeding also reduces problems with thermoregulation for the sow when she is restricted to the individual crate (Manteca et al. 2014).

Redirected behaviours are behaviours normal to the species but directed to an abnormal stimulus. These behaviours appear when animals are in an environment where an important stimulus is lacking, such as the materials and conditions to perform exploratory behaviour. When pigs are under these conditions, they direct this behaviour to what they have available, such as the bodies of their mates, e.g., tail biting or belly nosing.

These behaviours are also indicators of poor welfare, as animals are trying to cope with the environment, but they cope at some costs.

The measures recommended to reduce stereotypies and redirected behaviours are the provision of those tools necessary to allow the animals to perform as much as possible their natural behaviours, and especially their most important ones (behavioural needs).

2.2.5.5. Stress and fear from inadequate handling or a negative human-animal relationship:

Many years ago, Hemsworth and colleagues (2002) showed that chronic fear towards people, caused by negative handling from the stockpersons, consistently influences consistently the productivity of a farm. Many other authors have corroborated these results with different studies (Zulkifli 2013).

Directive 2001/88/EC obliges owners to employ staff with a foundation in animal welfare. Nevertheless, that does not always happen, and currently, animals still do not receive correct or positive treatment on many farms, which leads to chronic fear.

2.2.5.6. High culling rate of sows:

In general, the percentage of sows culled per year in an intensive farm is between 20 to 50% (Hadaš et al. 2015). That means 20 to 50% of the sows are sent to the slaughterhouse each year because they have not produced what it was expected by the breeder. The number of sows that have died are also included in this percentage.

Gilts that fail to grow or a gilt or a sow that produces a very small litter of piglets may have a welfare problem, often produced by the incorrect management of housing conditions, although other factors contribute to the wide variation in individual production (Broom and Fraser 2015). The problem lies in these sows being culled without an effort being made to solve the problem that creates this lack of productivity and other reproductive problems.

This issue is not addressed by the law because each breeder has the right to organise the culling rate of its farm as he/she wants.

2.2.5.7. Health problems of sows and pigs:

The most common health issues of the sows are urinary tract infections, traumatic injuries, broken bones, gastric ulcers and respiratory or enteric infections.

Under intensive conditions, sows are usually stressed for many reasons, and they use their adrenal cortex frequently. This constant activation impairs their immune system function, and as a result, they are more susceptible to diseases (Broom and Fraser 2015). For example: when sows are confined in individual crates, urinary infections increase because they have to lie on their faeces, their activity is almost inexistent, they drink less and urinate less than under other housing conditions, and all these conditions increase the apparition of urinary tract infections (Madec 1984).

Poor ventilation on a farm, which is very related to the ammoniac concentration, is not uncommon. High temperatures, especially in hot climates, with poor ventilation and a high density of animals, can considerably affect the health of pigs and their susceptibility to suffer some diseases, especially, respiratory diseases.

Directive 98/58/EC requires that all environmental conditions be kept within limits that are not harmful to the animals, but it does not specify the limits, so environmental conditions are not always acceptable or controlled.

2.2.5.8. Neonatal mortality:

High neonatal mortality is a significant animal welfare issue, as well as a productivity problem. In Europe, the neonatal mortality of an intensive pig farm, i.e., the mortality of piglets born alive, is between 11 and 13% (Kirkden et al. 2013), depending on the country and the farm. Thus, neonatal mortality it is still an important and unsolved problem.

The most common cause of neonatal mortality is crushing by their mother, which either kills them or debilitates them, so they are less able to suckle and more susceptible to suffering from disease. Crushing often happens with hypothermic and starved piglets, which are not able to escape crushing by the sow.

A decline in maternal behaviour also influences it, as sows are less responsive to the calls of the piglets being crushed. In addition, selection to increase the size of the litters negatively affects piglet mortality, as it results in a higher proportion of weaker piglets, with some piglets not having access to a teat for suckling, as more piglets than teats are often present (Andersen et al. 2011).

Selection by breeders has resulted in a substantial increase in sow size relative to that of her wild ancestor, but much less change has occurred in the birth size of the piglets, and a tendency has been observed for maternal behaviour to be impaired (Marchant et al. 2001).

Ways to address this problem through the law are not being contemplated, but it is an important animal welfare issue that remains unresolved.

2.2.5.9. Painful procedures in piglets and abrupt weaning:

Painful procedures (castration, tail docking) as well as weaning cause very difficult moments for piglets of some days of age. Castration and tail docking without anaesthesia and analgesia produce an acute and chronic pain, suffering, a high probability of suffering from infection (Lessard et al. 2002), and also, depending on the management and hygiene of the farm, a consequent high mortality.

These problems are treated by all the directives, but in an ineffective way, as currently, 80% of the farms in Europe continue to perform these painful procedures routinely, without anaesthesia or analgesia.

Weaning is a traumatic event for piglets as it is premature and abrupt and hence very different from natural conditions. These separations very negatively affect their welfare. One consequence is the increase of sucking and nosing behaviour to other piglets, which is known as belly-nosing, described as an up-and-down massaging movement with the snout placed under the belly of other pigs (Schmidt 1982). Another consequence of the weaning is fighting, which occurs at time when piglets are very susceptible, when the piglets from different litters are mixed, with the mixing further compromising their health.

The weaning procedure is also addressed by all the directives, allowing piglets to be separated from their mother at three weeks of age, even if it is recommended to do this later, when the piglets are at least four weeks of age.

2.2.5.10. Impossibility for the sow to move in most of the farrowing crates and individual crates:

This impossibility to move creates frustration and stress. The sow is going to be in a farrowing crate for 4 weeks and in an individual crate for approximately 4 more weeks, so they spend approximately 8 weeks during each cycle confined without being able to walk.

Some farrowing crates allow the sows to move until farrowing, as it is possible to remove the part that immobilizes the sow. Therefore, this type of crate (removable crate) is a little bit better for the sow's welfare.

Part of the discomfort, along with the sow's inability to move, is caused by thermoregulation problems because the sows in the crates cannot exchange body heat among each other, nor can they change their positions enough to regulate their body temperature. Furthermore, if the crates are not well designed, sows can suffer aggressions from other sows, without the possibility of escape.

As explained in the chapter on Ethology of pigs, sows are very motivated to perform nesting behaviour, which is considered a behavioural need. Even if we provide them a nest, they want to produce their own nest (Faucitano and Schaefer 2008), and they try to do it as much as they can. The current farrowing crates, where sows are housed for one week before farrowing until the moment of weaning, do not allow the performance of nesting behaviour, as the sow are neither able neither to move (to get far away from the other sows to be calm and alone) nor to create a nest with some material. In fact, when they are housed under these conditions, they change their posture frequently as well as performing movements that are similar to those performed during the construction of the nest. The consequences of the inability to perform this behaviour, is activation of the stress response, which increases the concentrations of plasmatic cortisol, beta-endorphins, as well as other hormones. Consequently, secretion of oxytocin would be reduced. This response will affect negatively with the farrowing, especially in primiparous and old sows, where a release of oxytocin is necessary for the farrow as well as for the colostrum ejection (Manteca et al. 2014).

Directive 120/2008/EC requires the provision of material to allow the sows to at least create a nest. Nevertheless, nesting is not usually accomplished because even if we provided the material, the sow would still not be able to move away, so the first part of the nesting behaviour would not occur.

2.3. Conclusion and transition:

In conclusion, actual pig production systems do not allow pigs to satisfy their fundamental needs. This inability has medical, environmental and ethical consequences, which influence both the welfare and productivity of pigs.

These welfare concerns lead to the next chapter on the study of animal welfare science; its relation with other important fields, such as productivity, food safety and public health; and the way to objectively measure animal welfare in pigs.

3. Animal Welfare Science:

3.1. Concept and evolution:

The concept of animal welfare is complex and variable depending of the perspective and the areas addressed, which include scientific, ethical, economic, cultural, political and legal arguments.

On a scientific basis there are three main approaches:

- The first approach emphasises the biological functioning of organisms, and one example of a definition is the one of Broom (1986), one of the most prominent scientists of animal welfare, who said: the welfare of an individual is its state as regards its attempts to cope with its environment; coping means having control of mental and bodily stability.
- The second approach emphasises that welfare is not simply the absence of stress, considering also emotions. One example of a definition is: welfare is a state of complete mental and physical health, where the animal is in harmony with its environment (Hughes 1976). Also, The Five Freedoms definition (Farm Animal Welfare Council 1979) corresponds to this approach.
- The third approach consists on the concept of "natural living": animals should be allowed to live according to their natural attitudes and behaviour, developing their natural adaptations (Carenzi and Verga 2009).

The first definition of animal welfare and the most universal one, even if with some limitations, is known as *The Five Freedoms*. Once the five freedoms are respected, a minimum state of welfare is provided to the animals:

- freedom from hunger and thirst, by providing readily accessible fresh water and a diet to maintain full health and vigour;
- freedom from discomfort, by providing an appropriate environment, including shelter and a comfortable resting area;
- freedom from pain, injury or disease, by preventing or rapidly diagnosing and treating;
- freedom to express normal behaviour, by providing sufficient space, proper facilities and company of the animal's own king; and
- freedom from fear and distress, by ensuring conditions and treatment to prevent mental suffering.

The Five freedoms (1979) were born out of an initial investigation on the welfare of farm animals kept in intensive production performed by professor Roger Brambell, in 1965. This investigation was organised because of huge public concerns about the quality of life of farm animals, after the publication of the book *Animal Machines* from Ruth Harrison (1964), and the posterior formation of the Farm Animal Welfare Council, in 1979, by the UK government. Thorpe, one of its members, emphasised that an understanding of the biology of the animals was important and explained that animals have needs with a biological basis, including some needs to show particular behaviours and that farmers would have problems if those needs were frustrated (Thorpe 1965).

The definition of the Five freedoms indicates that animal welfare includes the physical and mental state of the animal. Therefore, animals kept by humans must at least, be protected from unnecessary suffering.

Welfare can be measured scientifically and varies over a range from very good to very poor. Welfare will be poor when there is difficulty in coping or failure to cope. Various coping strategies involve behavioural, physiological, immunological and other components that are coordinated from the brain. Feelings, such as pain, fear and the various forms of pleasure, which are a key part of welfare, could be part of a coping strategy. As many coping strategies may be used in an attempt to cope with a challenge, a wide range of welfare measures may be needed to assess welfare (Broom 2011).

A key point of agreement amongst animal welfare scientists in the early 1990s was that animal welfare was measurable and hence a scientific concept (Broom 2011). During previous decades, acceptable animal welfare (called the minimum standard) has not only protected animals from unnecessary suffering, providing the ability to meet their needs, but has also provided animals with a life worth living, which consists of also taking into account an animal's positive experiences. Thus, it is not enough to prevent suffering from negative experiences, but it is essential to "experience" positive experiences, essential to mental state, which is one of the elements of animal welfare (Boissy et al. 2007; Fraser 2009; Farm Animal Welfare Council 2010).

Defining the concept of quality of life has taken on more importance during the last decade. Quality of life is a relatively new way of looking at farm animal welfare. Quality of life may be compromised by powerful economic and other forces that combine to determine an animal's collective experiences. To provide animals a good quality of life, not only is full compliance with the law necessary but also implementation of the good practices described in the Welfare Code is necessary: good welfare should be a main aim of husbandry with disease controlled by the strictest measures to ensure normal behaviour; availability of environmental choices and harmless wants; with a ban on most, if not all, mutilations; certain husbandry practices (including the manner of death) that are prescribed or forbidden; opportunities provided for an animal's comfort, pleasure, interest and confidence; and the highest standards of veterinary care, as well as the highest standard of stockmanship (Farm Animal Welfare Council 2010).

In 2012, the World Organisation for Animal Health adopted 10 'General Principles for the Welfare of Animals in Livestock Production Systems' to guide the development of animal welfare standards and research: (1) how genetic selection affects animal health, behaviour and temperament; (2) how the environment influences injuries and the transmission of diseases and parasites; (3) how the environment affects resting, movement and the performance of natural behaviour; (4) the management of groups to minimize conflict and allow positive social contact; (5) the effects of air quality, temperature and humidity on animal health and comfort; (6) ensuring access to feed and water suited to the animals' needs and adaptations; (7) prevention and control of diseases and parasites, with humane euthanasia if treatment is not feasible or recovery is unlikely; (8) prevention and management of pain; (9) creation of positive human–animal relations; and (10) ensuring adequate skill and knowledge among animal handlers (Fraser et al. 2013). Research directed at animal welfare, drawing on animal behaviour, physiology, veterinary epidemiology and other fields, complements the more established fields of animal and veterinary science and helps to create a more comprehensive scientific basis for animal care and management.

3.2. Relation to other fields:

3.2.1. Animal welfare and productivity and quality of the product:

Animal welfare is linked to productivity. First, if the welfare of the animals is poor, and we increase it, the productivity also increases. If we continue to increase productivity, the welfare would decrease, and vice versa. However, there is a point at which the productivity and animal welfare are high, without either being at their highest level. This point would be the optimum economic of welfare. If we increase the welfare past this point, the productivity would decrease, and vice versa.

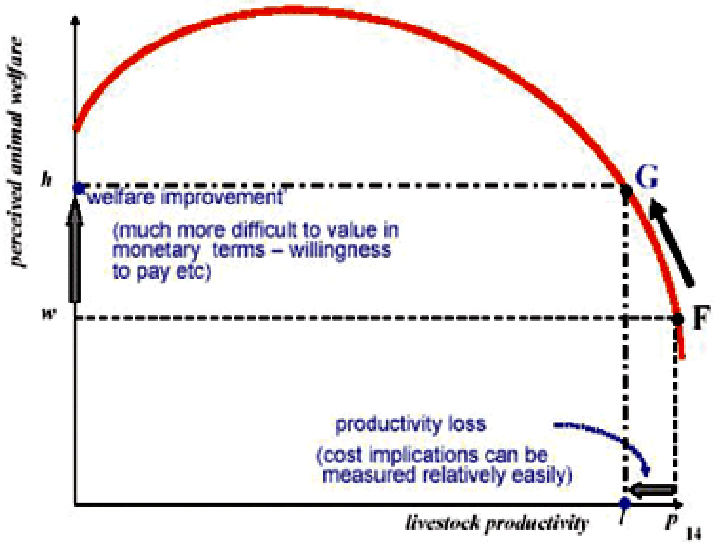

Figure 5: Productive loss as function of welfare increase and the ideal point that producer may be willing to reach while consumers may want to pay for it. Source: McInerney 2004.

Some states in which the welfare of animals is affected, directly influencing productivity, are fear, stress and health issues.

Fear is defined as a negative emotional state induced for the perception of a threating situation or associated to a potential threat (Boissy 1995). For instance, animals that are subjected to aversive human contact are highly fearful of humans and their growth and reproductive performance is compromised (Zulkifli 2013).

Stress is defined as the response of an organism in the presence of a situation of threat or that alters the homeostasis, the internal balance of the animal. This response is similar in all the species, and it includes physiological changes. First, activation of the sympathetic nervous system produces changes, such as an increase in heart rate; afterwards, the production of some hormones, such as glucocorticoids by the adrenal glands, induces more progressive changes, such as "mobilization of the glucose reserves of the organism". Stress also influences the behaviour of the animals, decreasing, for example, the appetite, and therefore decreasing the consumption behaviour, as well as reproduction behaviour, which also affects the productivity of the animals. These changes help the animal face to a threat situation, being a beneficial response for them. The problem is that, sometimes, this stress response persists, being prejudicial in this case for the animals, affecting their immune system, and making them susceptible to suffering infections and other diseases.

Stress caused by the housing and management of pigs may not only affect animal welfare but may also affect the acceptance of the product by the consumer as well as productivity (de Jong 2000). In addition, stress may also change the product quality (Figure 6). For example, stress caused by the mixing of unfamiliar pigs reduced the growth rate for weeks (Ekkel et al. 1996) and affected the meat quality (Warris et al. 1994).

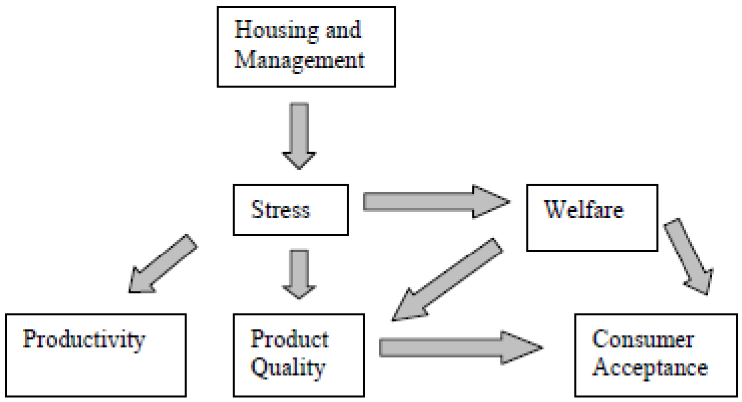

Figure 6: The relation between stress, caused by housing, management, welfare, productivity, product quality and acceptance of the animal products by the consumer. Source: Blokhuis et al. 1998.

Many years ago, the need for welfare assessment to consider all possible physiologic, immunologic, behavioural, anatomic, and agricultural performance indicators of stress and distress was stated (Curtis 1987). Furthermore, an examination of production measures alone was considered a dangerous practice, particularly when taking a herd, flock or system average (Fraser 1993). Furthermore, we should remember that animal welfare is about the welfare of an individual animal, not a system (Fraser and Broom 1990; Rollin 2002). Animal agriculture over the last few decades has intensified, and productivity has increased greatly. Does this mean that the animal welfare within the current systems has also increased greatly?

Thus, productivity and performance are linked to animal welfare, but they do not have a directly proportional relation. The difficulty is to find the way to ensure both.

3.2.2. Animal welfare and food safety/ public health:

Meat consumption has proven to be particularly vulnerable to safety issues, including Bovine Spongiform Encephalopathy (BSE), dioxin and hormone and veterinary drug residues (Verbeke 2001 and 2007). Indeed, by the end of the 1990s, meat had been described as the food item in which consumer confidence has decreased most (Becker 2000). Although major crises date back to several years, the Eurobarometer survey indicates that European consumers persist in expressing concerns about zoonoses, such as avian influenza (European Commission 2006).

In recent years, consumers have generally been uncertain about the safety and quality of their food. Their concerns about livestock production methodologies is increasing due to various outbreaks of food-borne zoonoses and animal diseases (Noordhuizen and Metz 2005). For pork production, a major issue has been that of bacterial contamination, in particular salmonellosis (Hurd et al. 2001; Boughton et al. 2007).

Today, consumers have quite an impact on animal production in Europe, especially regarding the husbandry system, animal health care and transport of animals. The European Commission has prioritized consumer protection in its policy, installed a precautionary principle, and created the European Food Safety Authority, EFSA.

Many reasons exist to indicate that a welfare-food safety link exists, which means that when we debate how animals should be treated, we are also debating the safety of our food. Nevertheless, food safety must be influenced by many other factors in addition to animal welfare.

In Europe, public health and food safety have become the most relevant drive relative to the production of food of animal origin. Animal health and welfare are following as a next priority, as they are very related to food safety. Safeguards in these areas are required, and product liability must be imposed. Farmers should show their farm status with respect to food safety and public health, as well as animal health and welfare (Noordhuizen and Metz 2005).

3.2.3. Animal welfare and ethical considerations:

European citizens care deeply about animal welfare, and in recent years, more and more people have become concerned about the ethical treatment of animals (European Commission 2012).

Thanks to the European Union's Lisbon Treaty, animals are currently recognised as sentient beings, meaning that they have the capacity to have feelings, including their ability to experience pleasurable states, such as happiness, and aversive states, such as pain, fear and grief (Broom and Fraser 2015).

General human opinion has evolved over time to encompass first all humans instead of only some humans, and afterwards. certain mammals that were kept as companions, animals that seemed most similar to humans, such as monkeys; larger mammals; all mammals; all warm-blooded animals; all vertebrates; and finally, some invertebrates (Broom and Fraser 2015). Nevertheless, in a practical way, these concepts are still not applied to the way we use and treat animals, as they are still considered to be a tool to satisfy human needs or wishes. A few current examples are the intensive production of farm animals, religious slaughter without stunning, some examples of severe animal experimentation, hunting as a sport, bull-fighting as a tradition, among others, all of which are legal in most countries.

Humans and other animals, especially social animals, have many biological mechanisms that enable them to behave in a moral way. It is not possible to live successfully in a social group unless the individuals have the ability to avoid harming others and perhaps to collaborate (Broom and Fraser 2015). Consequently, natural selection has favoured genes that promote abilities such as the recognition of individuals and the memory of moral and immoral actions (de Waal 1996; Ridley 1996; Broom 2003 and 2006), as well as empathy and compassion (Würbel 2009). These are the pillars of the first ethical considerations about the industrialization of the animal production in the 60s, when the book *Animal Machines* by Ruth Harrisson (1964) was published, creating a huge public reaction, and giving birth to the field of animal welfare.

Broom (Broom 2003, 2006b, 2010 and 2014b) stated that all human behaviour and laws should be based on the obligations of each person to act in an acceptable way towards other people and the animals with which they interact; if we use an animal in a way that gives us some benefit, we have some obligations to that animal; we have some obligations to any individual considered to have an intrinsic value. We should avoid causing poor welfare in the animal except where the action leads to a net benefit to that animal.

According to Morton (2010), recognizing the complexity of the ethical responsibilities throughout our interactions with animals is important. When a welfare problem is recognized, we are ethically obliged to do something about it. That is, we ought to minimize or abolish practices that result in poor animal welfare and promote those that deliver good welfare. We should provide a good quality of life for all animals (i.e., an overall balance of good over poor welfare throughout an animal's life), especially, if we are using them for our own benefits. Society expects the veterinary profession to have an informed opinion on how to promote good animal health and welfare (Morton 2010), which is why that field of research is encouraged.

However, acting on welfare concerns and implementing standards and legislation in a world market where trade agreements are becoming increasingly important is not always easy (Morton 2010). Moreover, animal welfare and ethics are strongly affected by national cultures, moral values, and the attitudes of those caring for animals (Morton 2010).

In terms of standards of welfare and legislation, when animals are suffering because of a sequence of events and when the suffering of the animals can be shown to be cause by these events, this suffering leads to ethical concerns that may subsequently be translated into standards and legislation. A procedure may then be made illegal, or at least, a requirement, or a guideline may be established to reduce or abolish the suffering. Trying to promote positive welfare in animals to achieve mental states such as happiness and contentedness is more difficult (Morton 2010). Thus, ethics is an important matter, very related to animal welfare science, and should be consciously taken into account (Broom 2011). In the end, it is the pressure of the society and its ethical concerns that can influence the law and the fields of research.

Morton (2010) stated that veterinarians are following in the footsteps of our medical colleagues in recognizing that mental health is a very important part of well-being and is essential to ensuring a good quality of life. In fact, the World Health Organization defined health as ''a state of complete physical, mental and social well-being and not merely the absence of disease". That means that an animal could be in a good physical health and free from disease but suffering mentally, e.g., separation anxiety in dogs, excessive confinement in some farmed and zoo animals, social isolation in laboratory animals, and so on (Morton 2010).

For this reason, research of how to assess positive emotions and positive welfare states seem essential to really ensure a good quality of life of farm animals, considering it as not only the absence of poor welfare but also the presence of positive welfare, including positive emotions.

3.3. Animal welfare assessment:

Animal welfare assessments should contain both physical and mental elements. The physical elements, such as behaviour, physiology, health, productivity and pathology, can be measured relatively easily, but the mental elements, such as the emotional states, remain much harder to quantify (Marchant-Forde 2009).

According to Broom (2000), some measures of welfare are physiological and behavioural indicators of pleasure: the extent to which strongly preferred behaviours can be shown, variety of normal behaviours shown or suppressed, extent to which normal physiological processes and anatomical development are possible, extent of behavioural aversion shown, physiological attempts to cope, immunosuppression, disease prevalence, behavioural attempts to cope, behaviour pathology, brain changes, body damage prevalence, reduced ability to grow or breed, and reduced life expectancy.

Some signs of poor welfare arise from physiological measurements. For instance, increased heart rate, adrenal activity, or reduced immunological response following a challenge, can all indicate that welfare is poorer than in individuals that do not show such changes (Moberg 1985). Care must be taken when interpreting such results because as with many other measures, crossover occurs with other parameters, such as behaviour, when better understanding the real welfare state of the animal.

Behavioural measures also have an important value in welfare assessment. For example, avoidance of an object or event by an animal gives much information about the animal's feelings and hence about its welfare; an individual that is completely unable to adopt a preferred posture when lying down despite repeated attempts will be assessed as having poorer welfare than one that can adopt the preferred posture; abnormal behaviours, such as stereotypies, self-mutilation, tail-biting, or excessively aggressive behaviour, indicate poor welfare (Broom 2008).

Health and performance could also be used as measures of welfare. Disease, injury, movement difficulties and growth abnormality indicate poor welfare. If two housing systems are compared in a controlled experiment, and the incidence of any of the above is significantly increased in one of them, that would mean that the welfare of the animals is worse in that system. For instance, Marchant and Broom (1996) found that sows in stalls had leg bones only 65% as strong as sows in group-housing systems. The actual weakness of bones means that the animals are coping less well with their environment, so welfare is poorer in the confined housing (Broom 2008).

As a result of differences in the extent of different physiological and behavioural responses to problems, any assessment of welfare necessarily should include a wide range of measures (Broom 2008).

As an example of a recent a complete animal welfare assessment of different species of farm animals, we have the Welfare Quality® project. This project proposes four welfare principles linked to 12 criteria (Figure 7) that result in good welfare (Blokhuis et al. 2010) and can be considered a useful guideline for achieving good welfare (Rushen et al. 2011).

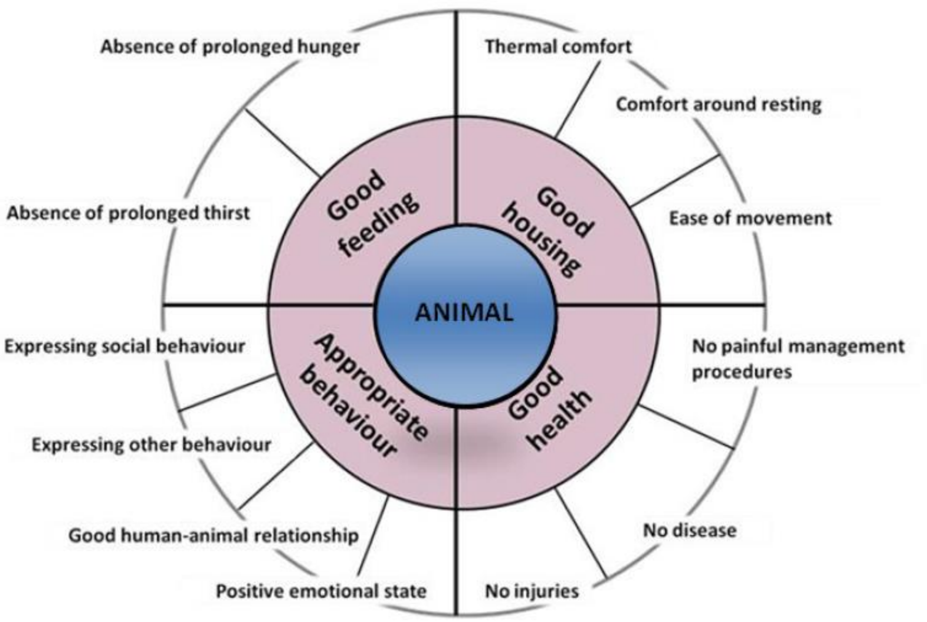

Figure 7: The four principles and 12 animal-based criteria used as guidelines for good welfare. Source: Welfare Quality ®, 2009.

According to the European Food Safety Authority (2012), two type of factors affect animal welfare: resource-based measures and management-based measures.

The first one refers to the physical environment and the availability of the resources, such as the space allowance, the housing conditions and the bedding material. The second one is related to the management practices of the farm, like, for instance, the use or not of anaesthetics and analgesics if mutilations are performed, the number of milking per day and so on. Both type of factors interact with each other, influencing the way they act on the animal.

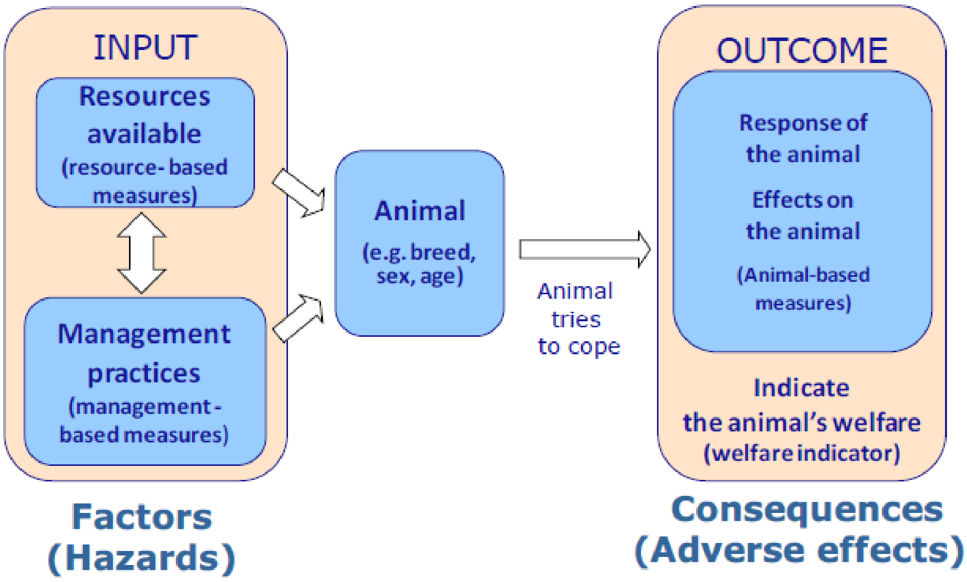

Figure 8: Input and outcome and their relationship. Source: European Food Safety Authority Journal, 2012.

In Figure 8, the types of factors that influence animal welfare and the way we can measure animal welfare are schematized: resource-based measures and management-based measures compose the inputs, which are the factors or hazards that directly influence the lives of the animal, as it tries to cope with its environment. To see the direct effect of this environment towards the animal, we can observe the response of the animals and the direct or indirect effects, which is known as animal-based measures, focused directly on the animal. Animal-based measures are the most appropriate indicators of animal welfare, and a carefully selected combination of them can be used to assess the welfare of a population of animals (European Food Safety Authority 2012).

The most appropriate combination of animal-based measures will depend on the purpose of the welfare assessment. In addition to selecting the most appropriate animal-based measures, more information is needed on the essential characteristics of the different measures (validity, reliability and feasibility) and thus the method to assess animal welfare.

Good welfare is now considered not only the absence of negative experiences but also the presence of positive experiences, such as pleasure. However, the scientific investigation of positive emotions has long been neglected, even as investigations have been increasing during the last decade. Some examples of potential indicators of positive emotions could be play, affiliative behaviours and some vocalizations in laboratory and farm animals under commercial conditions (Boissy et al. 2007).

In conclusion, further research is needed to investigate feasible indicators of positive animal welfare, including positive emotions, to improve the existent animal welfare assessments, while at the same time, promoting a better quality of life for animals.

3.3.1. Peripheral oxytocin as a potential indicator of animal welfare:

In agreement with some existing literature (Martin and Bateson 2007; Paul et al. 2005), we consider that emotional states can best be assessed by crossing behavioural and physiological measures. It may help us interpret the animal's affective state (Paul et al. 2005) and by extension, animal welfare (Boissy et al. 2007). According to literature (Boissy et al. 2007; Duncan 2005; Mellor et al. 2009), more research is needed to highlight indicators of emotional states in order to assess animal welfare in a better way (Freymond et al. 2014).

As possible physiological measures of positive welfare we selected peripheral oxytocin, because some authors suggested that it could be used as an indicator of positive welfare (Broom and Zanella 2004; Rault 2016).

Oxytocin is a nonapeptide hormone particularly involved in social behaviours, although it is also implicated in a variety of 'non-social' behaviours. Ishak et al. (2011) suggested that oxytocin induces a general sense of well-being because it is calming, improves social interactions, increases trust and reduces fear. Changes in oxytocin levels are linked to positive social experiences and decreased reactivity to stressful situations (Paul et al. 2005). Thus, some authors (Broom and Zanella 2004) suggested that plasma oxytocin could be used as a marker of well-being in mammals.

Rault (2016) showed that positive contact with humans leads to increased oxytocin concentration in pigs' cerebrospinal fluid. A positive human-animal relationship is important in animal husbandry, and several studies have shown that a negative relationships and fear of humans may increase chronic stress and decrease performance (Zulkifli 2013).

According to Rault (2017), given the increasing interest in affective states in psychology and animal welfare science, research on oxytocin as a potential indicator of positive animal welfare is encouraged.

3.3.2. Peripheral serotonin as a potential indicator of animal welfare:

Another potential indicator of animal welfare that we selected was peripheral serotonin, because it is involved in emotional states and linked to many behaviours related to animal welfare, according to the literature (Insel and Winslow 1998; Young and Moskowitz 2005).

The role of serotonin in behaviour is very complex and includes the regulation of mood and emotional states (Landsberg et al. 2013). Some authors suggested that serotonin may also promote calm, prosocial interactions, such as allogrooming in vervet monkeys and positive social interaction in young adult humans, both of which are positively associated with increased serotonin activity (Insel and Winslow 1998).

Young and Moskowitz (2005) suggested that serotonin has a role in promoting affiliative behaviours in human adults. Ursinus et al. (2013) showed that blood and brain serotonergic measures were related to pig behaviour during a novelty test as well as related to exploration after novel object drop. These results suggested a role of serotonin in biological responses that underlie the pigs' behaviour during challenging situations.

There are several studies showing an association between low levels of serotonin and aggressiveness in different species (Brown et al. 1989; Da Prada et al. 1988; Rosado et al. 2010). Nevertheless, other authors observed a positive correlation between cerebrospinal fluid levels of 5-hydroxyindoleacetic acid (5-HIAA, the main serotonin metabolite) and aggression (Van der Vegt et al. 2003). Some studies showed a negative correlation between cerebrospinal fluid 5-HIAA levels and aggression in primates (Higley et al. 1992; Westergaard et al. 2003). Other studies showed that a group of dogs with owner-directed aggression had lower levels of serum serotonin than a control group without aggression problems (e.g. Rosado et al. 2010). In addition, treatment with drugs that increase the serotonin levels reduces aggressive behaviour in hamsters (Ferris et al. 1999) and in dogs (León et al. 2006), and diets with low levels of tryptophan, which is the precursor of serotonin, cause an increase in aggressive behaviour (Young 1991). Finally, Amat et al. (2013) suggested that lower levels of serotonin would influence aggressive behaviour in dogs.

Mottolese et al. (2014) revealed an interaction between oxytocinergic and serotoninergic systems in the human brain, i.e., the role of oxytocin in the inhibitory regulation of serotonin. Other authors have shown that oxytocin exerts anxiolytic effects via the oxytocin receptor expressed in serotonergic neurons in mice (Yoshida et al., 2009). Finally, according to Eaton et al. (2012), oxytocin appears to have some organizational effects on serotonin innervation.

In conclusion, this monoamine (serotonin) was selected, even if it seems that is more linked to negative than positive welfare, because of some practical reasons, like the feasibility of their assay method (competitive immunoassay). Nevertheless, other neuromodulators could be more interesting for this subject, even if more difficult to measure, like the dopamine, as it is more linked to positive emotions and positive welfare (Knutson et al. 2002).

OBJECTIVES

The main objective of the thesis study was to investigate feasible new indicators of positive animal welfare in pigs.

More precisely, the aims were as follows:

- Investigate some physiological and behavioural indicators of positive welfare, instead of poor welfare, as there is a lack of indicators of positive welfare in farm animals; concerning the category of indicators, we decided to focus on physiology and behaviour, as they are more based on the animal (animal-based measures) than on the resources.

- Focus attention on emotions, which, although an important part of animal welfare, are not usually included in animal welfare assessments, as further research in the field is needed.

- Take in account the feasibility of the measures, as it is important in farm conditions.

- Start under controlled conditions with a model of domestic pigs (mini-pigs), and finish under real farm conditions with commercial pigs.

- Explore from some perspectives to include new indicators of emotions and positive animal welfare in farm animal welfare assessments, to improve them, as well as to prevent welfare problems at farms.

RESULTS

CHAPTER 1: Providing straw to allow exploratory behaviour in a pig experimental system does not modify putative indicators of positive welfare: peripheral oxytocin and serotonin

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1.1. Introduction:

A decision was made to start the first trials with mini-pigs, as a model of domestic pigs, for practical and ethical reasons (3Rs). The conditions of the trials were controlled, in an experimental setting, to control all the possible variables to ensure the validity of our results, and while trying to start with a good basis. The aim was to investigate potential physiological indicators of positive animal welfare, peripheral oxytocin and peripheral serotonin, according to the literature. To do that, an a priori context considered a positive welfare situation, straw provision, was organised in a group of mini-pigs, with the effect being compared to that of a control group. The short-term effect and the long-term effect of straw provision in the peripheral oxytocin and serotonin were analysed to see if the straw provision could influence the concentration of these neuromodulators, compared to that of the pigs that were not provided straw.

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Providing straw to allow exploratory behaviour in a pig experimental system does not modify putative indicators of positive welfare: peripheral oxytocin and serotonin

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Numerous studies have shown that providing straw to pigs can reduce undesirable behaviours such as aggression, tail biting and stereotypy. The measurement of various neuromodulators can be helpful in assessing the development of positive behaviours and overall animal welfare. The oxytocin release is frequently linked to positive emotions and positive welfare. It has been suggested that oxytocin modulates the serotoninergic system. This study aims to investigate the potential effect of straw provision in pigs on peripheral levels of oxytocin and serotonin. In total, 18 mini-pigs were involved in an exploratory study conducted in two parallel groups, Enriched (n = 10) and Control (n = 8) groups. Pigs were divided by group and housed in pens of two individuals. Straw was provided continuously only in Enriched group and renewed each day for 2 weeks. Two blood samples were drawn from each animal 5 to 10 min before providing the straw, and 15 min after providing straw, during the 1st week, to analyse peripheral changes in oxytocin and serotonin before and after straw provision, and determine the existence of a putative short-term effect. The same procedure was carried out for Control group, without straw provision. Long-term effects of straw provision were also examined using blood samples drawn at the same hour from each animal in the 2nd and 3rd weeks. During this time, animals had the permanent possibility to explore the straw in Enriched group but not in Control group. At the end of each week, one animal-keeper completed two visual analogue scales for each mini-pig regarding the difficulty/ease to work with and handle it and its trust in humans. Results showed peripheral oxytocin increases in both groups after 2 weeks (P = 0.02). Results did not demonstrate any effect of providing straw to allow exploratory behaviour on peripheral serotonin. Other results were not significant. This preliminary study explored the relationship between peripheral oxytocin and serotonin and the presence of straw that allow pigs to perform exploratory behaviour, suggesting that there was no relationship between them. Some future studies may include crossing oxytocin and serotonin with other parameters, such as behavioural measures, to obtain more information about the true state of the animal and any possible relationship with pig welfare.

Keywords: positive measures of animal welfare, oxytocin, serotonin, exploratory-rooting behaviour, behavioural needs

Implications

The main implication of this study was suggesting that it does not exist a relationship between peripheral oxytocin and serotonin and straw provision in pigs. Some studies evidenced that straw provision in pigs increases animal welfare. Other studies suggested that oxytocin and serotonin could be used to assess a positive welfare. Thus, we wanted to see if there was some association between the provision of straw and the changes in peripheral oxytocin and/or peripheral serotonin, showing no effect in our conditions. Further research is needed to see if peripheral oxytocin and/or serotonin could be considered as indicators of animal welfare.

Introduction

The standards of welfare we provide to animals should ensure a 'life worth living' (Farm Animal Welfare Council, 2009), and even a good quality of life (Duncan, 2005). Several authors therefore emphasised the importance of 'good welfare' defined as not only the absence of negative experiences or negative

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feelings but also the presence of positives experiences or positive feelings (Boissy *et al*., 2007). Almost all physiological measures used in welfare assessment tend to measure negative rather than positive affect under the assumption that a lack of negative affect is indicative of well-being. Nevertheless, some authors proposed that some neuromodulators, such as oxytocin and serotonin, could serve as indicators to assess animal welfare (Broom and Zanella, 2004; Boissy *et al*., 2007), and specifically a 'good welfare'. Also, measures of multiple physiological indicators coupled with observations of behaviour can be helpful in determining an animal's affective state (Paul *et al*., 2005) and thus, animal welfare (Boissy *et al*., 2007).

Oxytocin is a nonapeptide hormone well-known for its role in lactation and parturition. It is particularly involved in social behaviours, such as social memory, attachment, sexual and maternal behaviours, and aggression, although it is also implicated in a variety of 'non-social' behaviours, such as learning, anxiety, feeding and pain perception (Lee *et al*., 2009). Oxytocin administrations have been associated with decreased blood pressure and reduced reactivity to painful stimuli (Uvnäs-Moberg, 1998; Singer *et al*., 2008). Additionally, oxytocin promotes calmness, relaxation, growth and restoration (Uvnäs-Moberg, 1998). Ishak *et al*. (2011) considered that oxytocin induces a general sense of well-being because it is soothing, improves social interactions, increases trust and reduces fear, as well as inducing endocrine and physiological changes. Thus, Broom and Zanella (2004) suggested that all these oxytocin features may be linked to welfare and that plasma oxytocin may be used as a marker of well-being in mammals. In this regard, changes in oxytocin levels in relation to positive social experiences, associated with a decrease in sensitivity to stressful situations, have been observed (Uvnäs-Moberg, 1997 and 1998). Rault (2016) showed that positive human contact with animals led to an increase in the oxytocin concentration of cerebrospinal fluid in pigs over 120 min, and that the frequency of the positive interactions was correlated with the increase in oxytocin concentration. However, other research by Taylor *et al*. (2006) suggested that elevated plasma oxytocin may act as a signal for relationship distress in humans, in which case its function may be to motivate people to seek out positive social contacts. Further research is thus needed to investigate oxytocinergic system function in relation to welfare and stress states.

Some authors evidenced a link between brain oxytocin concentrations in pigs and positive interactions with humans (Rault, 2016). A positive human–animal relationship is important in animal husbandry, and several studies have shown that a negative human–animal relationship and fear of humans may increase chronic stress and decrease performance (Zulkifli, 2013). In addition, some studies have shown that oxytocin increases trust and reduces fear in humans (Ishak *et al*., 2011).

The role of serotonin in behaviour is very complex and includes the regulation of mood and emotional states, such as fear and aggression, arousal, impulse control, sleep–wake cycle, food intake and pain (Landsberg *et al*., 2013). Some authors propose that serotonin may also promote calm, prosocial interactions, such as allogrooming in vervet monkeys and positive social interaction in young adult humans, both of which are positively associated with increased serotonin activity (Insel and Winslow, 1998). Young and Moskowitz (2005) suggested that serotonin has a role in promoting affiliative behaviours in human adults in everyday life. This effect is probably not only a result of the ability of serotonin to inhibit aggressive responses but also a result of the ability of serotonin to facilitate prosocial behaviour.

Recently, Ursinus *et al*. (2013) showed that blood and brain serotonergic measures were related to pig behaviour during a novelty test, and that blood and brain serotonin were both related to exploration after novel object drop. These results suggest a role of serotonin in biological features, such as fear, underlying the behavioural responses of pigs when confronted with a challenging situation. It may help future research to understand the development of maladaptive behavioural or physiological responses in pigs (Ursinus *et al*., 2013). Assessing the relationship between behavioural responses and brain and blood serotonin parameters could help to form a better understanding of the development of maladaptive responses. Ursinus *et al*. (2013) also explored this line of research, using a test arena for pigs. The time spent exploring the test arena was significantly correlated with both platelet serotonin level and right hippocampal serotonin activity (turnover and concentration), leading them to conclude that the existence of an underlying biological trait may be involved in the pig's behavioural responses toward environmental challenges, and that this was also reflected in serotonergic parameters (Ursinus *et al*., 2013).

Recent animal studies demonstrated that specific links exist between oxytocinergic and serotoninergic system. Mottolese *et al*. (2014) revealed a form of interaction between these two systems in the human brain, that is, the role of oxytocin in the inhibitory regulation of serotonin. Eaton *et al*. (2012) stated that oxytocin has an organisational effect on the serotonin system.

In the present study, authors wanted to produce a context linked with a high level of welfare in pigs, according to the literature, to analyse some physiological effects. In the case of pigs, this could involve exploratory and rooting behaviour with manipulable material, like straw, which provides an ideal context for assessing welfare combining behavioural and physiological measures (Martin and Bateson, 2007).

The European Union's Commission Directive 2001/93/EC established that pigs must have permanent access to a sufficient quantity of material to enable proper investigation and manipulation activities. These materials are specified in the Council Directive 2008/120/EC, including straw, hay, wood, sawdust, mushroom compost, peat or a mixture of such, which do not compromise the health of the animals. The directive was developed with the intention of improving pig welfare by meeting the pigs' needs for exploration through the provision of rooting materials (Studnitz *et al*., 2007). Exploratory behaviour with manipulable material could be defined as rooting, chewing, sniffing and manipulating the available rooting material (Studnitz *et al.*, 2007). It appears to be a high priority behaviour in pigs, and numerous studies have shown that the provision of straw and other environmental enrichment can reduce undesirable behaviours such as aggression, tail biting (redirected behaviour consisting of biting the tail of their pen mates which could produce major injuries and a high mortality) and stereotypy (repetitive, invariant behaviour without apparent immediate function, caused by the animal's repeated attempts to adapt to its environment or by a dysfunction of the central nervous system) (Burbidge *et al.*, 1994). The provision of straw in animal production systems is widely presumed to be beneficial to animal welfare. There is weak evidence that concrete flooring rather than straw is a risk factor for increased overall morbidity and mortality (Tuyttens, 2005). The purpose of providing straw is to serve as a stimulus and outlet for exploratory and manipulative behaviour involving the snout and mouth, known as rooting behaviour (Tuyttens, 2005).

The aim of this study was to investigate whether the presence of straw and exploratory behaviour in pigs was associated with peripheral oxytocin and/or serotonin changes over time. These physiological measures may be associated to a situation of positive animal welfare, according to the literature, or a physiological change during a specific context linked to animal welfare, and further research is needed to be validated. Many authors have measured the efficiency of different types of enrichment material in pigs, using physiological indicators of animal welfare, and especially of bad or negative welfare (Tuyttens, 2005; van de Weerd and Day, 2009; Casal *et al.*, 2016). In our study, we wanted to study the physiological effects of a model of enrichment material in pigs (straw), focusing on the potential measures of positive welfare, instead of the negative ones. According to the studies about human–animal relationship mentioned above, we also assessed the animals' trust in humans and the difficulty or ease of working with them in regards of the concentrations of the neuromodulators herein studied, as well as investigating the differences in human–animal relationships between animals that have enrichment material and animals that have not.

Material and methods

The housing, husbandry and use of the animals for the procedures described in this article were carried out according to French and European legislation and in compliance with the principles of replacement, reduction and refinement. The project, including this experimental procedure, was approved by IRSEA's (Research Institute in Semiochemistry and Applied Ethology) Ethics Committee (no. 125) and the French Ministry of Research (AFCE_201602_02).

Animals and housing

The mini-pigs (*Sus scrofa domesticus*) ($n = 18$: castrated males = 8; females = 10) involved in the study were a new strain resulting from cross-breeding of miniature breeds (Asiatic potbelly breeds: Vietnamese and Chinese) with conventional white hair breeds (Landrace and Large White). The pigs were born and reared at the centre Specipig for breeding and biomedical research, in Barcelona, Spain. They entered into the present study at 7 months of age. They all have been housed, handled and managed in the same conditions during all their life. Animals were previously socialised to humans and habituated to the contention tools, such as the sling frame, for 3 months. Two pigs of the same sex and age were housed in each pen (2.5 m^2). Groups were created after weaning to avoid fighting, so pigs were used to be together. In one hall, they were four castrated males and six females, and in the other, four castrated males and four females. Pigs were housed in a controlled system in an experimental building designed for research, in two identical halls (30 m^2), with monitored environment parameters: mean ambient temperature of 22°C, same ventilation by 2 artificial ventilators in each hall in the same position and 60% of humidity. Pens were cleaned daily. Pigs were fed twice a day with commercial pig diets and had continuous access to drinking water. Lights were on from 0800 until 1800 h.

Procedure

At 7 months of age, 10 mini-pigs in one of the halls (Enriched group) received manipulable material to allow exploratory and rooting behaviour (Straw from Coustenoble, 1bd DEWAVRIN-BP 60044, Auchel, France). The pigs had no previous experience with straw; 20 min before the provision of straw, blood samples were taken (T_0) using a contention tool known as a sling frame, to which the mini-pigs had already been habituated. The time necessary for operators to remove the pig, put it into the sling frame, draw the blood by venepuncture from the jugular vein and return it to its pen was between 5 and 10 min. The sample by itself was obtained 5 min after the beginning of the procedure. Pigs were accustomed to the procedure: to go into the sling frame, to the contention method used for blood sampling and to the blood sampling procedure. After blood sampling, 5 kg of straw were provided to the pen (2.5 m^2), covering the entire surface of the floor, a video camera was switched on, and the exploratory session started. The exploratory session was defined as the first 10 min during which the pigs were exposed to the straw, during the 1st week. Performance of exploratory and rooting behaviour in pigs in 'Enriched group' was confirmed by video analysis, performing a continuous sampling by two independent observers (Table 1). A second blood sample (T_1) was drawn immediately after the exploratory session. After this, straw was continuously provided and renewed every day once for 2 weeks in each pen. In the other hall, 8 mini-pigs (Control group) were exposed to the same conditions regarding their management and the experimental methodology, but without straw. Blood samples were collected as in the Enriched group, at the same times. During the 2nd and 3rd week of the test, only one blood sample was drawn from the pigs (T_2 in the 2nd week and T_3 in the 3rd week), once a week for each pig, always in the same order, so that the interval between samples taken from each animal was always exactly 1 week. At the end of the 2 weeks, the straw was removed from the experimental pens and the test was complete.

Visual analogue scales
At the end of each week, one animal-keeper marked two visual analogue scales for each mini-pig regarding the difficulty/ease of work with and handle the pig (from 'animal-keeper has a lot of difficulty to work with and handle the pig' to 'animal-keeper does not have any difficulty to work with and handle the pig') and trust in humans (from 'the pig does not trust animal-keeper at all, and attempts to escape from him' to 'the pig trusts animal-keeper completely, and approaches him with confidence'). The degrees of the scale were from 0 to 10, having two opposite definitions: at 0 and the other one at 10. The length of the scale was 10 cm. The animal-keeper drew a perpendicular line in the visual analogue scale, which was measured and entered as a number from 0 to 10. The line indicates the perception of the animal-keeper in relationship with the definitions on the two extremities of the scale (Srithunyarat *et al.*, 2016). A total of two visual analogue scales of each type were completed for each mini-pig at the end of the test.

Physiological parameters
Blood collection. Blood samples were taken from the pigs from the jugular vein while pigs were in the sling frame. Prior to the test, all the pigs were subjected to a programme of habituation to handling that involved using the sling frame and blood sampling during a period of three months. Blood was collected in pre-chilled EDTA/K3 aprotinin tubes (BD Diagnostics, Elvetec, Meyzieu, France) for oxytocin assay and in tubes with a clot activator (VACUETTE GREINER; Alcyon, Paris, France) for serotonin assay. Blood samples were kept refrigerated at 4°C during the sampling period, and were then centrifuged at 1200 × **g** for 10 min at 4°C. Plasma or serum was recovered and stored at −18°C prior to analysis at IRSEA (Quartier Salignan, Apt, France).

Plasma oxytocin level. Oxytocin levels were measured by enzyme immunoassay according to the manufacturer's instructions (Oxytocin EIA kit; EnzoLifeSciences, Villeurbanne, France) after a C18 solid-phase extraction (HyperSep C18 SPE Cartridges, Thermofisher, Illkirch, France) allowing for a 10-fold increase in concentration (Bienboire-Frosini *et al.*, 2017).

Serum serotonin level. Serotonin levels were measured by enzyme immunoassay (Serotonin EIA kit, EnzoLifeSciences) according to the manufacturer's instructions. In this study on mini-pigs, the serum samples were diluted 1 : 50; this working dilution was first validated in the IRSEA laboratory by (i) confirmation of the parallelism between sample dilution curves and the standard curve, and (ii) evaluation of the mean precision and mean recovery on four validation samples, which were 9.6% and 90%, respectively. Measured values were multiplied by their dilution factor to obtain actual serum serotonin concentrations and expressed in ng/ml.

Statistical analysis
Data analysis was carried out using SAS 9.4 software Copyright© 2002-12 by SAS Institute Inc., Cary, NC, USA. The significance threshold was classically fixed at 5%. Homogeneity of the two groups at baseline (T_0 the 1st week) for oxytocin and serotonin levels was analysed according to Student's *t* test using the TTEST procedure. For serotonin levels, *t*-test was involved because normality and homogeneity of variances were established. For oxytocin levels, and because of non-normality of this variable, the non-parametric two-sample Wilcoxon's test was employed using the NPAR1WAY procedure.

For the analysis of short-term effects of straw provision on oxytocin concentration, because of a substantial individual variability between pigs and heterogeneity between the two groups at T_0, differences in oxytocin levels between T_1 and T_0 were computed and analysed. For serotonin, because of a substantial individual variability between pigs, differences in serotonin levels between T_1 and T_0 were computed and analysed. Differences between groups for $T_1 - T_0$ were verified according to Student's *t* test if normality and homogeneity of variances were established. If conditions were not established, a two-sample Wilcoxon's test was performed.

The long-term effect (2 weeks) of straw provision on oxytocin was evaluated by examining the change of oxytocin levels from T_2 to T_3. For serotonin, it was evaluated by examining the change of serotonin levels between T_0, T_2 and T_3. Data were tested from the assumptions of normality using the residual diagnostics plots and the UNIVARIATE procedure. Homoscedasticity was tested by Levene test using the GLM procedure. If normality was established and variances were homogeneous: a GLM was used to evaluate effect of group, time and group × time interaction using the MIXED procedure. If normality was not verified, other distributions would be tested. If normality was verified but not homoscedasticity, the group = option (it defines an effect that specifies heterogeneity in the covariance structure of R) would be used in the mixed procedure. If normality was not verified but another distribution was identified, GLM would be used through the GLIMMIX or the GENMOD procedure depending on distribution identified.

For oxytocin level, normality was not verified. γ Distribution was identified as the one followed by oxytocin, which was consequently modelled by generalised estimating equation using the GENMOD procedure to evaluate effects of group, week, and group × week interaction. Because of heterogeneity at T_0, T_0 data were removed from main effects of the model but included it as a covariate, according to the literature (Committee for Medicinal Products for Human Use, 2015). This methodology generally improves the efficiency of analysis and avoids conditional bias from chance covariate imbalance. Repeated measures ANCOVA was used to analyse data without this bias. The best structure of variance–covariance to apply to data was chosen by minimising QIC criteria. For serotonin level, normality was verified, and GLM was used to evaluate effect of group, week and group × week interaction, with the MIXED procedure. But, as the variances between the two groups were heterogeneous (heteroscedasticity), the group = group option was added in the repeated statement to specify a heterogeneity in the covariance structure of R. The best structure of variance–covariance to apply to data was chosen by minimising AIC and BIC criteria. For oxytocin and serotonin, *post hoc* multiple comparisons were done using the Tukey–Kramer adjustment.

'Difficulty/ease to work with and handle pigs' and 'Trust in humans' visual analogue scales followed Poisson distribution. To evaluate effect of group, week and group × week interaction, 'Trust in humans' visual analogue scale was modelled by using the GLM with the GLIMMIX procedure as recommended. For the 'Difficulty/ease to work with and handle pigs' visual analogue scale, the generalised estimating equation with the GENMOD procedure was used because no suitable model was available using the GLIMMIX procedure. *Post hoc* multiple comparisons were done using the Tukey–Kramer adjustment.

Results

First, it has been confirmed by video recording that pigs performed exploratory behaviour during exploratory sessions (Table 1). Before the provision of straw, groups were heterogeneous at T_0 for oxytocin values ($Z = -2.68$; $P = 0.01$). For serotonin values groups were homogeneous at T_0 ($t = -0.02$; $P = 0.99$).

Table 1 *Raw data of exploratory session during 600 s in Enriched group*

Subjects	Exploratory behaviour frequency	Exploratory behaviour duration (s)
1	1	598
2	1	599
3	2	598
4	2	599
5	2	599
6	1	599
7	1	599
8	1	600
9	1	600
10	1	600

Short-term effect of exploratory behaviour on oxytocin and serotonin: 1st week

For $\Delta(T_1 - T_0)$ model, no significant difference was found between 'Control' and 'Enriched' groups (−0.63 ± 2.91 *v.* 0.63 ± 3.73 pg/ml; $Z = -0.09$; $P = 0.93$). Regarding serotonin, for $\Delta(T_1 - T_0)$ model, no significant difference was found between 'Control' and 'Enriched' groups (−101.85 ± 804.29 *v.* −147.35 ± 1181.84 ng/ml; $t = 0.09$; $P = 0.93$).

Long-term effect (2 weeks) of exploratory behaviour on oxytocin and serotonin: 1st, 2nd and 3rd week

Regarding oxytocin, the difference between groups was not significant ($P = 0.17$) (Table 2). A significant difference was observed between weeks (3.90 ± 2.78 *v.* 6.16 ± 2.72 pg/ml; $P = 0.02$) (Table 3). More precisely, an increase of oxytocin concentration means of both groups between T_2 and T_3 was found significant (6.16 ± 2.72 *v.* 3.90 ± 2.42 pg/ml; $P = 0.02$). The group × week interaction was significant ($P = 0.05$) (Table 4). Even if the difference between the two groups was not significant, descriptive data showed that the increase in oxytocin concentration was more representative in the Enriched group than in the Control group (Table 4).

Concerning serotonin, there was no significant difference between 'Control' and 'Enriched' groups (2162.24 ± 661.02 *v.* 2278.44 ± 1333.14 ng/ml; $P = 0.68$ (Table 2). There was also no significant difference of serotonin values of both groups between weeks ($P = 0.13$) (Table 3). The group × week interaction was not significant ($P = 0.35$) (Table 4).

Visual analogue scales

There was no significant difference between 'Control' and 'Enriched' groups regarding the difficulty/ease to work with and handle the pigs (8.07 ± 1.88 *v.* 7.52 ± 2.65; $P = 0.39$) (Table 2), or their trust in humans (7.67 ± 2.37 *v.* 7.12 ± 2.93; $P = 0.59$) (Table 2). No differences were observed between the 3 weeks ($P = 0.23$ and $P = 0.14$) (Table 3) and for the group × week interaction ($P = 0.31$ and $P = 0.50$) for the two visual analogue scales, respectively (Table 4).

Discussion

The main aim of this study was to investigate whether straw provision in pigs, allowing them to perform exploratory behaviour, was associated with peripheral oxytocin and/or

Table 2 *Comparisons between groups (Control group and Enriched group) for all the parameters*

	Descriptive statistics (mean ± SE)		Analysis		
Group…	Control	Enriched	Value	df	*P*-value
Oxytocin level	6.54 (0.59)	3.66 (0.37)	1.87	1	0.17
Serotonin level	2162.24 (134.93)	2278.44 (247.56)	0.18	1	0.68
Trust in humans VAS	7.67 (0.50)	7.12 (0.54)	0.30	1	0.59
Difficulty/ease to work with and handle pigs VAS	8.07 (0.39)	7.52 (0.49)	0.74	1	0.39

VAS = visual analogue scale.
Values are mean (SE) for oxytocin level, serotonin level, trust in humans VAS and difficulty/ease to work with and handle pigs VAS.
No significant differences were observed.

Table 3 *Comparisons between the different weeks for all the parameters: 1st (baseline T_0, before straw provision), 2nd and 3rd week*

	Descriptive statistics (Mean ± SE)			Analysis		
Week…	1 (T_0 baseline)	2	3	Value	df	*P*-value
Oxytocin level	4.90 (0.70)	3.90 (0.59)[a]	6.16 (0.66)[b]	5.31	1	0.02
Serotonin level	2168.94 (223.59)	2086.99 (257.24)	2433.04 (292.73)	2.20	2	0.13
Trust in humans VAS	6.71 (0.84)	8.54 (0.37)	6.97 (0.54)	2.03	2	0.14
Difficulty/ease to work with and handle pigs VAS	7.37 (0.64)	8.63 (0.27)	7.40 (0.62)	2.97	2	0.23

VAS = visual analogue scale.
Values are mean (SE) for oxytocin level, serotonin level, trust in humans VAS and difficulty/ease to work with and handle pigs VAS.
[a,b]Values with unlike superscript letters correspond to a significant difference ($P = 0.02$).

Table 4 *Comparisons of Control group and Enriched group according to the different weeks for all parameters*

	Descriptive statistics (Mean ± SE)								
	Control			Enriched			Analysis		
Week…	1 (T_0 baseline)	2	3	1	2	3	Value	df	*P*-value
Oxytocin level	6.58 (1.02)	5.63 (1.06)[c]	7.31 (1.02)[e]	3.21 (0.48)	2.69 (0.34)[df]	5.14 (0.75)	3.94	1	0.05
Serotonin level	2164.93 (248.72)	2086.72 (258.54)	2235.07 (221.77)	2172.15 (362.61)	2087.20 (428.25)	2609.02 (526.54)	1.09	2	0.35
Trust in humans VAS	7.80 (1.04)	8.10 (0.72)	7.15 (0.83)	5.83 (1.23)	8.88 (0.34)	6.83 (0.75)	0.71	2	0.50
Difficulty/ease to work with and handle pigs VAS	7.98 (0.93)	8.21 (0.45)	8.05 (0.61)	6.88 (0.89)	8.96 (0.31)	6.88 (1.01)	2.33	2	0.31

VAS = visual analogue scale.
Values are mean (SE) for oxytocin level, serotonin level, trust in humans VAS and difficulty/ease to work with and handle pigs VAS.
[c,d,e,f]Values with unlike superscript letters correspond to significant differences ($P = 0.01$ and $P < 0.0001$, respectively). The multiple comparisons have been computed using the LSmeans statement using the adjustment of Tukey–Kramer.

serotonin changes. We first confirmed by video analysis that pigs performed exploratory behaviour after the provision of straw. Then, we found that peripheral levels of oxytocin and serotonin in pigs were not modified by straw provision in the context of this study. Besides, human–animal relationship between groups was assessed thanks to two visual analogue scales, showing that there was no effect of the provision of straw on human–animal relationship in our test conditions.

The outcome of this study was divided into two parts: short-term effect (immediately after the first provision of straw) and long-term effect (after 2 weeks where straw was provided continuously) of the provision of straw as a model of enrichment material in pigs. Regarding the short-term effect of both oxytocin and serotonin (the difference between T_0 and T_1, so before and after the provision of straw), we did not observe any significant difference between groups nor over time; it thus appears that straw, and so the possibility to perform exploratory and rooting behaviour, does not have an impact on oxytocin and serotonin in a short-term situation.

Fries *et al.* (2005) stated that the half-life of oxytocin in plasma is very short (between 3 and 10 min), and Ludwig and Leng (2006) showed that, in rodents, the secretion of endogenous oxytocin triggered by the intraperitoneal injection of hypertonic saline appeared as a peak in plasma in the first 30 min. So, the little literature about this topic suggested that the time chosen to blood sample in our study was the correct one, 10 to 20 min after 'Exploratory session' (the first 10 min of straw). Nevertheless, we cannot be sure that our only blood sample could show a possible peak of peripheral oxytocin or serotonin due to a specific event in a particular moment, even if it was necessary to try to do it in that way, according to the literature, before starting with more invasive procedures. The next step, after obtaining a negative result using that way, would be to perform further blood samples with a catheter, to see if the same result is obtained.

A significant result regarding plasma oxytocin concentration on the long term was observed. More precisely, the mean level of oxytocin in both groups during the 3rd week was significantly higher compared with the 2nd week. That result suggested that the presence of straw (allowing exploratory and rooting behaviour) had no effect on peripheral oxytocin because the mean of the two groups showed a significant increase in plasma oxytocin between the 2nd and 3rd week but not between the 'Control' and 'Enriched' groups. This increase could be explained by an effect of handling. During the trial, all animals were indeed in contact with humans, and particularly with operators that they were accustomed to working with after a long period of socialisation and habituation. All animals had greater contact with them during the study than under normal conditions, and this could trigger an increase of plasma oxytocin levels because of a positive human–animal relationship (Rault, 2016). The fact that the increase of oxytocin seems to be higher in Enriched group between the 2nd and the 3rd week than in Control group, even if not significant, could give rise to an interesting question: could the peripheral oxytocin keep increasing in 'Enriched' group if the provision of straw was longer, for example 1 or 2 more weeks? For this reason, the authors think that further studies focalised in a longer follow-up would be interesting. Finally, the results may suggest that oxytocin could be only affected by social interactions or social enrichment, and not by individual positive states, like the allowance to perform exploratory behaviour with enrichment material. Thus, other neuromodulators than oxytocin and serotonin could be explored.

Regarding peripheral serotonin, no differences were found between groups nor between group × time, so we may assume that there is no effect of the provision of straw allowing exploratory behaviour on serotonin, at least, in our test conditions. Most animal studies focusing on behavioural responses in an experimental test, measure serotonin during or directly after the behavioural test, implying that they measure a state the animal was in at that particular moment (Ursinus *et al.*, 2013). However, for practical reasons, the same blood sampling was used to assay both oxytocin and serotonin but the sampling schedule was mainly driven by plasma oxytocin features. Thus, as explained for the short-effect of the provision of straw in oxytocin, it is difficult to know if the blood sample performed 10 to 20 min after 'Exploratory session' (the first 10 min of straw), could show a peak of serotonin, making very difficult to see the effect of a specific event in a particular moment. Besides, Ursinus *et al.* (2013) suggest that the relations found in their study between behaviour and measures of serotonin in blood and brain indicate an underlying (personality) trait rather than states that vary in time.

This study also allows us to perform some preliminary observations about human–animal relationship, thanks to the visual analogue scales of difficulty/ease to work with and handle the pigs and their trust in humans. The observations showed that the provision of straw did not produce a significant difference regarding trust in humans nor in the ease of working with the animals. Contrarily, some studies have shown that the provision of environmental enrichment could improve human–animal relationship, so, their trust in humans (van de Weerd and Day, 2009). It could also improve the ease to work with animals and handle the pigs (van de Weerd and Day, 2009). Other studies showed an association between oxytocin and a positive human–animal relationship (Romero *et al.*, 2014; Rault, 2016). However, if we take into account our conditions, it is important to mention that the individual personality of each animal could influence this result (Wemelsfelder, 2007), and the fact that the animals were highly socialised to humans during a long period prior to the study. Thus, our study suggests that the provision of straw does not influence the human–animal relationship, when it is already very positive. Positive human × animal interactions also have been documented to play a role in sustaining the welfare and production of domestic animals (Caroprese *et al.*, 2006; Zulkifli, 2013), hence, we consider that it is an interesting point to continue exploring.

It is also important to more carefully examine the way in which peripheral oxytocin is measured in pigs. Oxytocin is a neurohormone that, instead of favouring volume or synaptic transmission following central neuropeptide release, it follows a more dynamic concept with multiple and variable modes of release and communication. This concept considers neuropeptides in the extracellular fluid of the brain rather than those in the cerebrospinal fluid or plasma as primary signals, triggering a variety of receptor-mediated effects, including those underlying behavioural and neuroendocrine regulation and psychopathology (Landgraf and Neumann, 2004). In the current study, for practical reasons, it was decided to measure oxytocin by blood samples that were always drawn at the same time from all the animals, immediately before and following the provision of straw, and once a week for 2 more weeks during the continuous provision of straw. Paul *et al.* (2005) stated that the interpretation of some parameters, like oxytocin, is complicated by the fact that a particular measure could reflect both a positive or negative emotional state. For example, an increase in locomotory activity or in heart rate can be both associated with escape from predation (negative) and with sexual behaviour (positive). Often the method of collection for biological samples, like blood sampling in our trial, can cause emotional reactions in animals that may confound the results (Broom and Johnson, 1993). Some studies in rabbits showed that certain stressful events are linked to a peripheral oxytocin increase (Noller *et al.*, 2013). Other factors could influence the level of oxytocin, such as substantial inter-individual variations (Crockford *et al.*, 2014) as well as its short half-life of ~10 min in plasma (Fries *et al.*, 2005). Hence, taking all this information into account, a single measure of peripheral oxytocin at a specific time, perhaps, may not be a very sensitive measure by itself. For future studies, it would be interesting to analyse oxytocin several times during the day and over a longer period to evaluate how levels vary over time. The feasibility of taking these measures with a catheter should be explored in further studies in pigs.

One difficulty has been found regarding the fact of obtaining higher basal oxytocin values in the Control group in week 1, compared with the Enriched group, before having the straw. To explain that fact, the following hypothesis is proposed: the existence of individual differences is proved due to many reasons, like individual personality (Olff *et al.*, 2013) and it could influence the hormonal baseline. Apart from that, the statistical method could face this situation, as explained above, so we could measure both short-term effect and long-term effect without any bias, and that is a situation which could be frequent in physiological studies.

Studying oxytocin and other hormones in relation to straw provision and social interactions in pigs opens the door to improve the measurement of these parameters and its feasibility. As Rault *et al.* (2017) state recently, it is premature to judge oxytocin's potential as an animal welfare indicator given the few and discrepant findings and lack of standarisation in methodology, so research in that field is necessary to add information about this topic. In that respect, some of us have recently detailed and validated a method to assay plasma oxytocin in seven domesticated species, that has been used here, aiming at providing reliable and standardized measurement tools to researchers in this field (Bienboire-Frosini *et al.*, 2017). The findings of this study can serve as a foundation to further discussion about the interest of measuring these parameters, and about how best to study them in relation to the existing literature.

In conclusion, this preliminary study explores the relationship between peripheral oxytocin and serotonin and the presence of straw that allow pigs to perform exploratory behaviour, suggesting that there is no relationship between them. Nevertheless, some future studies may include crossing oxytocin and serotonin with other parameters, such as behavioural measures, to obtain more information about the true state of the animal and any possible relationship with pig welfare.

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Declaration of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics statement

The project, including this experimental procedure, was approved by IRSEA's (Research Institute in Semiochemistry and Applied Ethology) Ethics Committee (n°125) and the French Ministry of Research (AFCE_201602_02).

Software and data repository resources

None.

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1.3. Discussion:

Straw provision did not affect the peripheral oxytocin and serotonin, even though, after two weeks, oxytocin began to increase, though not significantly, in the straw group. Future studies of longer duration would be interesting to conclude whether straw really does not affect these neuromodulators or if it has an effect when straw provision occurs for a longer term, such as one month or more. For that reason, we decided to try to investigate the effect of a positive situation that seemed to have, a priori, a more direct and quicker effect, such as the provision of some toys, to see if these two modulators could be related to a positive welfare state or not.

CHAPTER 2: Selection of putative indicators of positive emotions triggered by object and social play in mini-pigs

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2.1. Introduction:

The present study was very similar to the preceding one, but instead of providing straw, we provided another type of enrichment material—some medium-sized dog toys—on which the mini-pigs performed object play behaviour. The behaviour indicated another type of positive context for the pigs, according to the literature, but was more punctual. Thus, we aimed to investigate the effect of toy provision on peripheral oxytocin and serotonin as potential indicators of positive animal welfare. In addition, this time, we included some preliminary observations of behaviour, as it is essential to use different types of indicators to assess animal welfare and to understand the state of the animals.

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ABSTRACT

Positive welfare is more than the absence of negative experiences. Nevertheless, there are few feasible animal-based measures for positive welfare. This study aimed to investigate whether object and social play behaviours in pigs, which is believed to trigger positive emotions, is linked to peripheral oxytocin and/or serotonin changes over time. Moreover, the study examines the relationship between tail movement, play behaviour, and peripheral levels of oxytocin and serotonin in animals that have the possibility to play, along with the approach towards humans and the difficulty/ease of working with the animals. At six months old, 10 mini-pigs from the Play group participated in one or two play sessions per day for three weeks. Eight mini-pigs from a Control group did not participate in play sessions. For each first play session, blood was sampled before the play session (T_0) and 5–10 min after the play session ended (T_1). The same procedure was performed each day for three weeks. For the Control group, blood samples were drawn at the same times as the experimental group, the following day. Results showed a Play session effect on oxytocin, where a significant difference was observed for time (Chi-Square = 3.88, DF = 1, p = .05) and for group*time interaction (Chi-Square = 5.65, DF = 1, p = .02): in the Control group, T_1 was significantly higher than T_0 ($p < 0.01$). Regarding Play session effect on serotonin, a significant difference was observed for time (Chi-Square = 5.92, DF = 1, p = .02), between T_0 and T_1 in the mean of both groups, but there was no significant difference between groups nor between group*time. No significant difference was found for the long-term effect of toy provision on oxytocin and serotonin. There was no significant difference between Play group and Control group regarding the difficulty/ease of working with and handling the pigs and their approach towards humans in our test conditions. Finally, some interesting correlations were found in the Play group during week three: positive correlations between object play frequency and tail movement duration (r = 0.84), as well as between social play duration and tail movement duration (r = 0.60); and a negative correlation between the motivation to play and the increase of peripheral serotonin between T_0 and T_1 ($r = -0.66$). This study investigated the effect of toy provision in a pig experimental system on peripheral oxytocin and serotonin, as well as other parameters. The results demonstrate some interesting effects, which could be further studied for use as potential physiological and behavioural measures of positive emotions in pigs.

1. Introduction

Beyond the absence of negative experiences, a positive state of welfare may exist when the health, nutritional, environmental, behavioural, and mental needs of animals are met. This occurs when negative states are absent and when positive states are present (Mellor et al., 2009). According to Duncan (2005), the lack of data on positive emotional states opens new areas of investigation for the assessment of farm animal welfare.

Play behaviour is a commonly observed, characteristic behaviour of young mammals (Bekoff and Byers, 1998). It is a likely candidate to indicate positive affective states and positive emotions (Mellor et al., 2009). Among all its functions, play enables animals to develop flexible kinematic and emotional responses to unexpected events in which they experience a sudden loss of control (Špinka et al., 2001). Play can improve welfare through self-reward, positive experiences, or social bonding (Sommerville et al., 2017).

Reimert et al. (2013) suggested that tail movement could serve as a behavioural indicator of positive emotions in pigs: both tail wagging and tail posture changes occurred more often during rewarding than

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aversive events. Other studies have also reported tail wagging in pigs to be related to positive situations such as social greeting (Kiley-Worthington, 1975; Terlouw and Porcher, 2005) and play (Newberry et al., 1988). Reefmann et al. (2009) have shown that tail movements may be an indicator of positive welfare in other species, such as sheep.

In agreement with some existing literature (Martin and Bateson, 2007; Paul et al., 2005), we consider that emotional states can best be assessed by crossing behavioural and physiological measures. It may help us interpret the animal's affective state (Paul et al., 2005) and by extension, animal welfare (Boissy et al., 2007). According to literature (Boissy et al., 2007; Duncan, 2005; Mellor et al., 2009), more research is needed to highlight indicators of emotional states, and particularly of emotional valence, in order to assess animal welfare in a better way (Freymond et al., 2014). In the present study, authors wanted to produce a context linked with a high level of welfare in pigs according to the literature, and to analyse some physiological effects. In the case of pigs, as mammals, this could involve play behaviour, which provides an ideal context for assessing welfare combining behavioural and physiological measures. As possible physiological measures of positive welfare we selected peripheral oxytocin, because some authors suggested that it could be used as an indicator of positive welfare (Broom and Zanella, 2004; Rault, 2016), as well as peripheral serotonin, because it is involved in emotional states and linked to many behaviours related to animal welfare (Insel and Winslow, 1998; Young and Moskowitz, 2005).

Oxytocin is a nonapeptide hormone particularly involved in social behaviours, although it is also implicated in a variety of 'non-social' behaviours. Ishak et al. (2011) suggested that oxytocin induces a general sense of well-being because it is calming, improves social interactions, increases trust and reduces fear. Changes in oxytocin levels are linked to positive social experiences and decreased reactivity to stressful situations (Paul et al., 2005). Thus, some authors (Broom and Zanella, 2004) suggested that plasma oxytocin could be used as a marker of well-being in mammals.

Recently, Rault (2016) showed that positive contact with humans leads to increased oxytocin concentration in pigs' cerebrospinal fluid. A positive human-animal relationship is important in animal husbandry, and several studies have shown that a negative relationships and fear of humans may increase chronic stress and decrease performance (Zulkifli, 2013). In line with this research and in addition to the effects of enrichment materials, the present study also assessed the approach towards humans and the difficulty/ease of working with them in regards to neuromodulator concentrations.

The role of serotonin in behaviour is very complex and includes the regulation of mood and emotional states (Landsberg et al., 2013). Some authors suggested that serotonin may also promote calm, prosocial interactions, such as allogrooming in vervet monkeys and positive social interaction in young adult humans, both of which are positively associated with increased serotonin activity (Insel and Winslow, 1998). Young and Moskowitz (2005) suggested that serotonin has a role in promoting affiliative behaviours in human adults. Recently, Ursinus et al. (2013) showed that blood and brain serotonergic measures were related to pig behaviour during a novelty test as well as related to exploration after novel object drop. These results suggested a role of serotonin in biological responses that underlie the pigs' behaviour during challenging situations.

Mottolese et al. (2014) revealed an interaction between oxytocinergic and serotoninergic systems in the human brain, i.e., the role of oxytocin in the inhibitory regulation of serotonin. Other authors have shown that oxytocin exerts anxiolytic effects via the oxytocin receptor expressed in serotonergic neurons in mice (Yoshida et al., 2009). Finally, according to Eaton et al. (2012), oxytocin appears to have some organizational effects on serotonin innervation.

The purpose of this study was to investigate whether the opportunity for play through the presence of toys was associated with peripheral oxytocin and/or serotonin changes in pigs over time. Moreover, it analysed the relationship between other potential measures of positive emotions in pigs, such as tail movement, with play behaviour and peripheral levels of oxytocin and serotonin, as well as the approach towards humans, and the difficulty or ease of working with and handling the animals.

2. Material and methods

The housing, husbandry and use of the animals involved in this experiment were carried out according to French and European legislation and in respect of the principles of replacement, reduction and refinement. The project, including this experimental procedure, was approved by IRSEA's (Research Institute in Semiochemistry and Applied Ethology) Ethics Committee (n°125) and the French Ministry of Research (AFCE_201602_01).

2.1. Animals and housing

The mini-pigs *(Sus scrofa domesticus)* (n = 18: CM = 8; F = 10) involved in the study were a new strain resulting from cross-breeding of miniature breeds (Asian potbelly breeds: Vietnamese and Chinese) with conventional white hair breeds (Landrace and Large White), born and reared at the centre Specipig for breeding and biomedical research, in Barcelona, Spain. The pigs were entered into this study at six months of age and they had never been involved in other studies. Animals were previously socialised to humans and habituated to the restraining tools, such as the sling frame, and blood sampling during a period of three months. Two pigs of the same sex and age were housed in each pen (2.5 m^2). Groups were created after weaning to avoid fighting, so pigs were used to be together. Pigs were housed in a controlled system in an experimental building designed for research, in two identical halls (30 m^2), with monitored environment parameters: mean ambient temperature of 22 °C, same ventilation by 2 artificial ventilators in each hall in the same position and 60% humidity. In one hall, there were 4 castrated males and 6 females, and in the other, 4 castrated males and 4 females. Pens were cleaned daily. Pigs were fed twice a day with commercial pig diets and had continuous access to drinking water. Lights were on from 8.00 a.m. until 6.00 p.m.

2.2. Procedure

At six months of age, 10 mini-pigs from one hall (Play group) participated in a 10-min video-recorded play session in the morning, on the day of the test, and a 30-min play session every afternoon, from Monday to Friday for three weeks. The 8 mini-pigs in the other hall (Control group) did not participate in play sessions. During Play sessions, pigs were placed in their own pens (2.5 m^2 on concrete floor), where two medium-sized dog toys (balls, ropes or balls with ropes) were introduced. The test started when one operator put the toys on the floor in the centre of the pen and then exited the pen. The type of toy (always the same for all the animals) was changed each week in order to solicit greater attention from the pigs. In each pen, two toys were provided in order to reduce conflict from competition. Behavioural events were video recorded during play sessions (only in Play group), to confirm that pigs played during play sessions (Tables 1 and 2). During the third week, behaviours were scored from video using continuous recording with an ethogram by two independent observers. Prior to each play session, faeces and urine were removed from the pen. Tests were carried out five days per week for three weeks, using the pigs from two pens each day for a total of four animals per day. Each pig performed the test once a week (on Monday, Wednesday, and Friday for the animals in the Play group; on Tuesday and Thursday for the animals in the Control group). In order to increase play time, all of the pigs in the Play group participated in unrecorded play sessions in the afternoon throughout the three weeks.

Table 1
Ethogram used for video analysis of Play session.

Behaviour	Definition
Object play/interaction with toy	Animal manipulates the toy or securely holds it in its mouth, energetically shaking it or carrying it around the pen (Newberry et al., 1988).
Social play	Two individuals interact in synchronisation, playing together with or without the toy, pushing the opponent with the head or shoulder or nudging (gentle snout contact) (Chaloupková et al., 2007).
Tail movement/tail wagging	Tail swinging in any direction, but mostly from side to side (Reimert et al., 2013).
Agonistic behaviour for competition	Actively displacing another pig, ramming or pushing another pig with the head with or without biting, aggressively biting any part of another pig or actively pursuing another pig (Chaloupková et al., 2007).

The frequencies and durations of each type of behaviour were analysed.
Behaviours could be overlapped, not mutually exclusive.

Table 2
Raw data of 600 s Play session.

Subject	Week	OPF	OPD	SPF	SPD	TMF	TMD	ABF	ABD
1	1	11	514	4	96	13	393	0	0
2	1	1	471	1	19	9	368	0	0
3	1	8	504	15	270	10	156	1	2
4	1	14	594	15	296	17	269	0	0
5	1	8	493	8	274	12	460	1	6
6	1	11	457	10	282	11	479	2	10
7	1	14	596	11	203	6	505	0	0
8	1	15	562	12	138	13	434	0	0
9	1	12	594	12	316	7	525	0	0
10	1	8	584	11	250	6	556	0	0
1	2	11	503	7	76	15	473	0	0
2	2	9	581	7	63	14	330	0	0
3	2	11	540	5	70	17	316	0	0
4	2	8	482	7	79	6	515	0	0
5	2	9	462	5	63	6	541	1	1
6	2	8	405	4	66	6	292	1	2
7	2	10	600	6	186	5	578	0	0
8	2	9	549	5	181	22	319	0	0
9	2	7	598	3	80	3	563	0	0
10	2	7	591	3	70	1	591	0	0
1	3	7	600	5	56	5	533	0	0
2	3	8	599	6	78	13	298	0	0
3	3	5	600	0	0	15	303	0	0
5	3	10	598	9	181	9	514	1	3
6	3	11	578	9	132	8	472	1	3
7	3	11	600	11	197	8	496	1	2
8	3	12	513	10	143	7	463	1	3
9	3	11	579	6	149	6	568	0	0
10	3	9	569	10	145	4	577	0	0

OPF: Object play frequency.
OPD: Object play duration.
SPF: Social play frequency.
SPD: Social play duration.
TMF: Tail movement behaviour frequency.
TMD: Tail movement behaviour duration.
ABF: Agonistic behaviour frequency.
ABD: Agonistic behaviour duration.

2.3. Visual analogue scales

During the play session, in the Play group, two operators marked a visual analogue scale for each mini-pig regarding its motivation to play: from 'the pig does not play at all and it does not have any interest in playing with the toy during the play session' to 'the pig plays a lot and shows a lot of interest in playing with the toy during the play session'. At the end of each week, one animal keeper marked two visual analogue scales for each mini-pig regarding the difficulty/ease of working with and handling the pig (from 'animal-keeper has a lot of difficulty working with and handling the pig' to 'animal-keeper does not have any difficulty working with and handling the pig') and its approach towards humans (from 'the pig does not approach the animal-keeper at all, it attempts to escape from him' to 'the pig completely approaches the animal-keeper with confidence'). Similar tests have been used in pigs by some authors (Hemsworth et al., 1999). The scales ranged from 0 to 10, with the two extremities of the scale (0 and 10) having opposite definitions. The length of the scale was 10 cm. The animal-keeper drew a perpendicular line in the visual analogue scale, which was measured and entered as a number from 0 to 10. The line indicated the perception of the animal-keeper in relationship to the definitions on the two extremities of the scale (Srithunyarat et al., 2016; Sutton et al., 2013). A total of 3 visual analogue scales of each type were completed for each mini-pig at the end of the test (Fig. 1).

2.4. Physiological parameters

2.4.1. Blood collection

Blood samples were taken from the pigs' jugular vein at 6 months of age while pigs were in the sling frame. Before the test, all the pigs were subjected to a structured program of handling and habituation to the sling frame, as well as to blood sampling, during a period of three months, in order to reduce the stress of the procedure as much as possible. The first sample was taken 30 min before the play session (T_0) and the second one 5–10 min after the play session (T_1). T_0 was considered as the 'baseline' for the peripheral level of oxytocin and serotonin, and T_1 was used to measure the direct effect of Play sessions on the physiological parameters in the Play group. Blood samples were taken at the same time of the day (from 9.00 a.m. to 12.00 a.m.) for all the pigs: on Monday, Wednesday, and Friday in Play group, and on Tuesday and Thursday in the Control group. This procedure was performed for three weeks, in order to study the long-term effect of toy provision on peripheral oxytocin and serotonin, comparing the T_0 of the three weeks between groups. Blood was collected in EDTA/K3 aprotinin tubes (BD Diagnostics, Elvetec, Meyzieu, France) for the oxytocin assay and in tubes containing a clot activator (VACUETTE GREINER, Alcyon, Paris, France) for the serotonin assay. Blood samples were kept refrigerated at 4 °C during the sampling period and were then centrifuged at 1200 × g for 10 min at 4 °C. Plasma was recovered and stored at −18 °C prior to analysis at IRSEA.

2.4.2. Plasma oxytocin level

Oxytocin levels were measured by enzyme immunoassay according to the manufacturer's instructions (Oxytocin EIA kit, EnzoLifeSciences, Villeurbanne, France) after C18 solid-phase extraction (HyperSep C18 SPE Cartridges, Thermofisher, Illkirch, France) allowing for a 10-fold increase in concentration. This procedure was previously validated (Bienboire-Frosini et al., 2017).

2.4.3. Serum serotonin level

Serotonin levels were measured by enzyme immunoassay (Serotonin EIA kit, EnzoLifeSciences, Villeurbanne, France) according to the manufacturer's instructions. This kit has been previously employed using piglet serum (Willemen et al., 2012). In this study on mini-pigs, the serum samples were diluted 1:50. This working dilution was first validated in the IRSEA lab by (i) confirmation of the parallelism between sample dilution curves and the standard curve, (ii) evaluation of the mean precision and mean recovery in 4 QC samples, which yielded results of 9.6% and 90% respectively. Measured values were

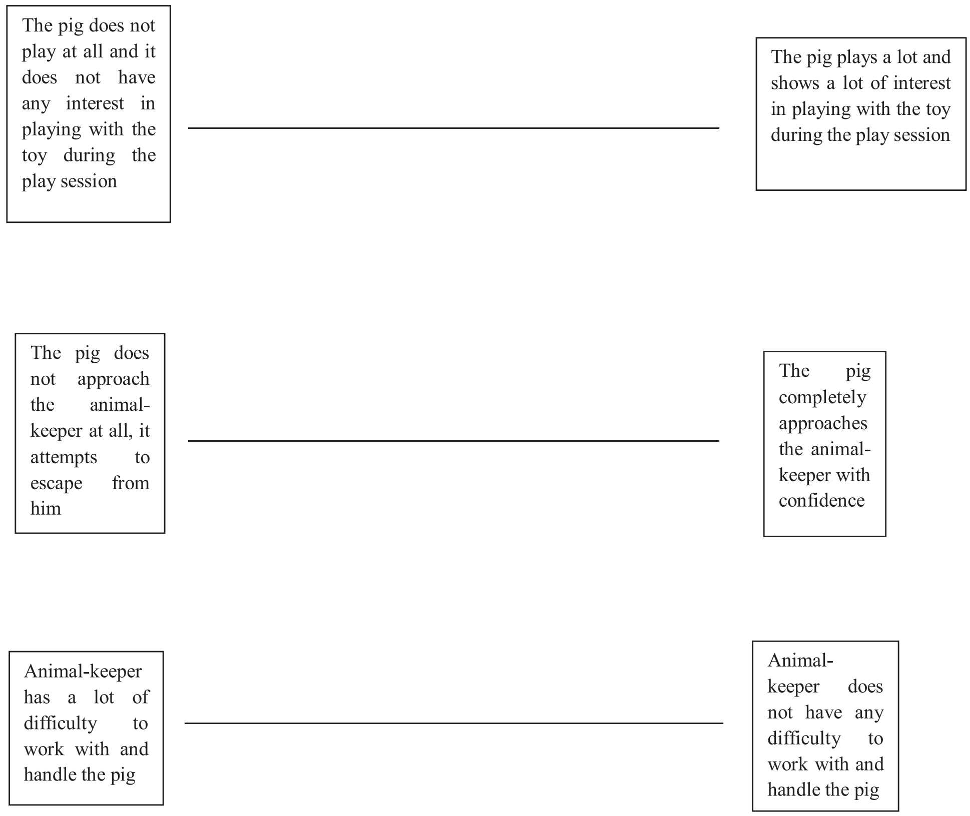

Fig. 1. Visual analogue scales.

multiplied by their dilution factor to obtain actual serum serotonin concentrations expressed in ng/ml.

2.5. Statistical analysis

Data analysis was carried out using SAS 9.4 software Copyright (c) 2002–2012 by SAS Institute Inc., Cary, NC, USA. The significance threshold was classically fixed at 5%. Homogeneity of the two groups at baseline (T_0 the first week) for oxytocin and serotonin levels was analysed according to Student *t*-test using the TTEST procedure for serotonin level, because normality and homogeneity of variances were established. Because normality was not established for oxytocin level, the non-parametric two-sample Wilcoxon test was carried out using the NPAR1WAY procedure.

Concerning oxytocin and serotonin, two different models were performed for each one. The first assesses the play session short-term effect on peripheral oxytocin and serotonin. To do so, a mixed model was performed to evaluate the effect of group (Play and Control Group), week (1, 2 or 3) and time (T_0 and T_1). Data were tested from the assumptions of normality using the residual diagnostics plots and the UNIVARIATE procedure. Normality was not established, and oxytocin and serotonin were identified as following a gamma distribution, which was consequently modelled by the Generalized Estimating Equation using the GENMOD procedure. The best structure of variance-covariance to apply to data was chosen by minimising QIC criteria.

The second model assesses the long-term effect of play sessions on oxytocin and serotonin concentration. The long-term effect of toy provision on oxytocin and serotonin was evaluated by observing the changes in oxytocin and serotonin levels at T_0 during the three weeks of the trial. Data were tested from the assumptions of normality using the residual diagnostics plots and the UNIVARIATE procedure. Homoscedasticity was tested by Levene's test using the GLM procedure. For serotonin levels, conditions were respected and the General Linear Mixed model was used to evaluate effect of group, week, and group*week interaction, using the MIXED procedure. The best structure of variance-covariance to apply to data was chosen by minimising AIC and BIC criteria. For oxytocin level, normality was not established. Oxytocin was identified as following a gamma distribution which was consequently modelled by the Generalized Estimating Equation using the GENMOD procedure to evaluate effect of group, week, and group*week interaction. The best structure of variance-covariance to apply to data was chosen by minimising QIC criteria. For oxytocin and serotonin, post-hoc multiple comparisons were done using the Tukey-Kramer adjustment.

Visual scales for 'Difficulty/ease of working with and handling the pigs' and 'Approaching humans' followed Poisson distributions and were modelled using the Generalized Linear Mixed Model thanks to the GLIMMIX procedure, in order to evaluate effect of group, week, and group*week interaction. Post-hoc multiple comparisons were done using the Tukey-Kramer adjustment.

Correlations between visual analogue scales, oxytocin, serotonin, and behaviours of the third week were carried out with the help of the CORR procedure, regarding the Pearson correlation coefficient, when variables followed a normal distribution, or the Spearman correlation coefficient when variables did not follow a normal distribution.

3. Results

3.1. Oxytocin and serotonin levels in Play and Control groups

3.1.1. Play session short-term effect on oxytocin

A significant difference was observed for time (Chi-Square = 3.88, DF = 1, $p = .05$) and for group*time interaction (Chi-Square = 5.65, DF = 1, $p = .02$). More precisely, in the Control group, T_1 was significantly higher than T_0 ($p < .01$) (Fig. 2).

3.1.2. Play session short-term effect on serotonin

A significant difference was observed for time (Chi-Square = 5.92,

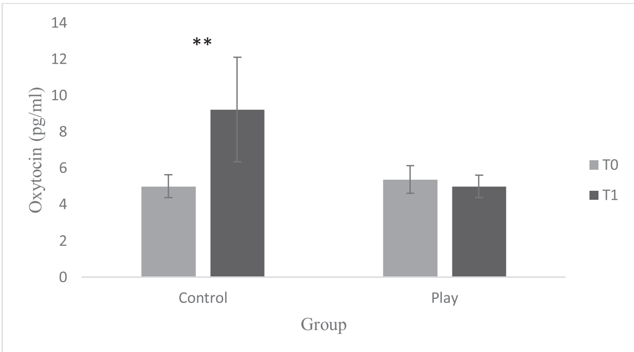

Fig. 2. Play session effect on oxytocin (pg/ml): effect of group * time. Mean ± Standard error. ** $p < .001$.

DF = 1, p = .02). More precisely, T_1 was significantly higher than T_0 in the mean of both groups. There was no significant difference between groups nor between group*time.

3.1.3. Long-term effect of toy provision on oxytocin and serotonin

Differences between oxytocin values at T_0 over the 3 weeks were not significant. The same result was obtained for serotonin. There was thus no evidence of a long-term effect of play on oxytocin or serotonin (Table 3).

3.2. Visual analogue scales

There was no significant difference between Play group and Control group regarding the difficulty/ease of working with and handling the pigs and their approaching towards humans (Table 4).

3.3. Correlations of Play group during the 3rd week (long-term effect)

In the Play group, after two weeks of play sessions, a positive correlation was found between object play frequency and tail movement duration (r = 0.84), as well as between social play duration and tail movement duration (r = 0.60); a negative correlation was found between the motivation to play (VAS of Play session) and the increase of peripheral serotonin between T_0 and T_1 (r = −0.66).

4. Discussion

The main aim of this study was to investigate whether toy provision in pigs for the purpose of soliciting play behaviour was associated with peripheral oxytocin and/or serotonin changes. We first confirmed by video analysis that pigs performed play behaviour after toy provision. We subsequently found that peripheral levels of oxytocin differed between the Control group and the Play group, but no effect of toy provision on peripheral serotonin was found. In addition, the influence of human-animal relationships between groups was assessed using to two visual analogue scales, which showed no effect of toy provision on human-animal relationship in our test conditions. Finally, some correlations were found in the Play group, such as the duration of tail movement behaviour and object play frequency, as well as social play duration.

Table 3
Long-term effect of toys provision on oxytocin and on serotonin.

Neuromodulator	Week	Group	Mean of T_0	Std Dev
Oxytocin (pg/mL)	Week 1	Control	5.20	0.62
		Play	5.60	4.20
	Week 2	Control	4.41	3.61
		Play	6.55	3.15
	Week 3	Control	5.24	3.55
		Play	3.69	2.49
Serotonin (ng/mL)	Week 1	Control	1914.56	580.32
		Play	1408.29	611.50
	Week 2	Control	1646.03	333.38
		Play	1772.23	871.10
	Week 3	Control	1622.58	298.67
		Play	1758.79	975.52

Table 4
Visual Analogue Scales of 'Approaching humans' and 'Difficulty/ease of working with and handling the animals'.

Visual Analogue Scale	Week	Group	Mean (Score 0–10)	Std Dev
Approaching humans	Week 1	Control	3.47	3.93
	Week 2		3.77	3.17
	Week 3		5.50	3.23
	Week 1	Play	5.41	3.42
	Week 2		4.26	3.19
	Week 3		4.41	3.62
Difficulty/ease of work	Week 1	Control	8.26	3.23
	Week 2		7.31	3.23
	Week 3		9.03	0.91
	Week 1	Play	8.06	1.59
	Week 2		7.70	2.74
	Week 3		8.56	3.07

Regarding the short-term effect of the play session on oxytocin, an increase in peripheral oxytocin was found in the Control group, but not in Play group. The Play group demonstrated more stable levels of oxytocin after the play sessions and after blood sampling. This interesting result suggests that animals in the Play group may already be in a balanced state due to the presence of environmental enrichment and the opportunity to play, unlike the animals of the Control group, who demonstrated an increase in plasma oxytocin following two blood sampling events which could activate a need to cope. Despite a prior procedure of habituation aimed to reduce the stressfulness of the blood sampling, it is possible that this handling could still trigger an increase in plasma oxytocin level to dampen the HPA axis activation (Noller et al., 2014; Wotjak et al., 1998). Noller et al. (2013) have already made a similar observation in rabbits. On another hand, both groups benefited from social interactions with the operators during the handling (stroke, reward). Some studies in other species have shown that oxytocin increases after intra or interspecies social interaction (Handlin et al., 2011; Odendaal and Meintjes, 2003). Rault (2016) also showed that positive human contact with pigs led to an elevation in oxytocin concentration in the cerebrospinal fluid. However, this was not observed in the Play group in our study. It is possible that pigs that play are less sensitive to that human-animal interaction because they may already be in a positive state of emotions due to the play sessions and the social play with a congener.

Scollo et al. (2014) stated that the interpretation of some parameters, like oxytocin, is complicated by the fact that a specific measure could reflect both a positive or negative emotional state. Often the method used for the collection of biological samples, such as blood sampling in our study, can cause emotional reactions in animals that may confound the results (Broom and Johnson, 1993). This result suggests that the balance or stability of oxytocin in an individual rather than its increase following an event (either positive or negative) could be a measure of animal welfare. As Rault et al. (2017) stated recently, it is premature to judge oxytocin's potential as an animal welfare indicator given the few and discrepant findings and lack of standardisation in methodology; further research is necessary to expand our understanding of the subject. In that respect, we have recently developed and validated a method to assay plasma oxytocin in seven domesticated species. It has been used here with the aim of providing reliable and standardised measurement tools to researchers (Bienboire-Frosini et al., 2017).

In terms of the short-term effect of play sessions on serotonin, an increase was observed in both groups, leading us to conclude that the effect was due to handling during blood sampling, in both groups. Ursinus et al. (2013) suggest that serotonin plays a role in biological traits, such as fearfulness, underlying the behavioural responses of pigs during a challenging situation. It thus seems that a stressful event such as blood sampling could have a stronger effect on peripheral serotonin than the possibility to play, resulting in an effect for all the animals rather than the Play group alone.

Regarding the long-term effect (three weeks effect) of toys provision on oxytocin and serotonin, the question was whether providing toys to pigs during three weeks for at least one 30-min session per day, could modify the basal level of oxytocin and/or serotonin. Results showed no effect of play on oxytocin or serotonin after the three weeks of the study. One explanation could be that the short duration and frequency of the stimulus (only one 30-min session per day plus a 10-min session on the day of the test) may not be sufficient to affect peripheral oxytocin or serotonin in a long-term evaluation. Another hypothesis is that only certain forms of play affect oxytocin release. We have evidence that social interactions trigger oxytocin release, but there is currently no evidence that object play affects oxytocin. In our study, as it is difficult to organise social play in animals (because of its spontaneous nature), the type of play chosen was object play, even if social play also appeared during play sessions. Another explanation could be that peripheral oxytocin and serotonin are not affected by play. It would be interesting for future studies to develop other methodologies, with more sessions per day, to gain more information about this parameter over a long-term period. It may also be interesting to perform a study with social play behaviour and its link to peripheral oxytocin and serotonin.

Analysis of the visual analogue scales showed that providing toys in the pens to encourage play was not sufficient to produce a significant difference between groups regarding approaching animal-keepers and the difficulty/ease of working with the pigs. However, it is important to mention that the individual personality of each animal could influence this result (Wemelsfelder, 2007), and also the fact that the animals were highly socialised to humans during a long period prior to the study. Some studies have shown that the provision of environmental enrichment could improve human-animal relationships and thus their approach towards humans and the ease of working with and handling them (Rodarte Covarrubias et al., 2005). Nevertheless, other studies suggest that enrichment-objects do not affect the ease of handling the pigs (Day et al., 2002; Hill et al., 1998). That is why, van de Weerd and Day (2009) suggest that the type of enrichment and quantity of stimuli provided influences the extent of the effect on behaviour towards humans. Human-animal interactions also have been documented to play a role in sustaining the welfare and production of domestic animals (Zulkifli, 2013). This possible relationship was not observed in our test conditions.

Regarding the correlations of physiological and behavioural measures in the Play group, during the third week, some interesting associations were found. For tail movement duration, we found a positive correlation with object play frequency and with social play duration. These results suggest that the more a pig plays, the more it moves its tail. According to Reimert et al. (2013), our results suggest that tail movement behaviour could be a new potential behavioural indicator of positive emotions in pigs. Our study sheds more light on the topic, and supports their hypothesis. Further research would be warranted to explore more thoroughly tail movement in pigs and its link with play and other activities.

Another correlation was found regarding peripheral serotonin and the motivation to play: more precisely, a negative correlation was found between the motivation to play (VAS of Play session) and the increase of peripheral serotonin between T_0 (before play session) and T_1 (after play session). It suggests that, the more a pig is motivated to play, the smaller the increase in serotonin after playing (or after handling and blood sampling). This preliminary result is interesting because it seems to suggest that animals that are very motivated to play with the toys, playing continuously during the 10-min session, could cope better with a challenging situation. This effect could be represented by a less pronounced increase in serotonin or even no increase after blood sampling. Further results are needed to explore the positive and negative effects of handling and challenging situations on peripheral serotonin, as well as the relationship between play behaviour and peripheral serotonin.

As we know, play behaviour is considered to produce positive emotions (Mellor et al., 2009), and much research has suggested that it can also be considered as an indicator of animal welfare (Boissy et al., 2007; Brown et al., 2015; Donaldson et al., 2002; Held and Špinka, 2011; Mintline et al., 2013). In the context of animal welfare, there has been increasing interest in the concept of positive welfare (Yeates and Main, 2008). Emotions and mood are contagious in pigs, so promoting play behaviour in some individuals can improve the welfare of group-housed animals (Held and Špinka, 2011; Reimert et al., 2013). Our results suggest that pigs that are allowed to play are more capable of coping with stressful situations, and this is the first definition of animal welfare. The study of approaching humans and the ease or difficulty of working with and handling the pigs is also interesting for real pig production systems. Our results present potential physiological parameters, such as oxytocin and serotonin, to be measured with behavioural indicators, such as tail movement, as indicators of a positive state.

5. Conclusions

These findings about putative indicators of positive emotions triggered by play in mini-pigs may prove important for assessing and improving animal welfare in production systems. The duration of tail movement behaviour in a group of pigs could be used as an easy and feasible behavioural indicator of positive emotions. Stable oxytocin levels in pigs could also indicate the animals' ability to cope with different situations that normally occur during their lives in farms, and could thus be considered as a potential physiological indicator of welfare in pigs. Further research is required to learn more about these possible measures of animal welfare in experimental and controlled systems, and their implications and applications in pig production systems.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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2.1. Discussion:

The present study suggested that peripheral oxytocin could be considered a potential indicator of positive emotions in pigs, even if further research would be required for confirmation. Apart from that, the study provided us much more information, as it allowed us to confirm that it is difficult to try to measure a positive welfare by blood samples because, even if the stress is reduced as much as possible, whether the effect is due to the blood sample or due to the treatment that you applied, or both together is difficult to determine. Therefore, a decision was made to introduce some potential behavioural parameters into the study, allowing us to obtain preliminary correlations that could guide us as we continue in this field of research. The inclusion of behavioural parameters for study together with physiological parameters seemed interesting, and the two measures were intersected with the aim of obtaining more information about the real state of the animal.

CHAPTER 3: Tail and ear movements as possible indicators of emotions in pigs

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3.1. Introduction:

This study focused more on the behaviour and potential behavioural indicators. We also went more in depth in the analysis of the emotional state of the animals, as an important part of welfare. We used the same situation, a play situation, as it worked very well in the preceding study, as a model for a situation that would evoke positive emotions. As potential behavioural indicators of positive emotions, according to the literature and according to our preliminary results, we used tail and ear movements.

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Tail and ear movements as possible indicators of emotions in pigs

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ABSTRACT

A better understanding of animal emotions is an important goal in disciplines ranging from neuroscience to animal welfare science, but few reliable tools exist for measuring these emotions. Play behaviour is generally recognized as a trigger of positive emotions in mammals, and previous studies have suggested that tail movement (a behaviour often seen during play) may indicate positive emotions in pigs, while ear movements may indicate negative emotions, or a decrease in positive emotions. This study investigated whether the provision of toys to solicit play behaviour in pigs was associated with tail and ear movement changes, with the goal of confirming the use of these movements as indicators of different emotions in pigs. Sixteen mini-pigs were divided into two identical rooms of 8 individuals. During 4 consecutive days, each animal participated in the study for a total of two days in two different situations: one Control session and one Play session. Each animal served as its own control. During Play sessions, pigs were placed in their own pens of two individuals, where two medium-sized dog toys were introduced. During Control sessions, animals were placed in their own pens, but no toy was provided. Behaviours were scored from video using continuous recording with an ethogram, which also allows to confirm that pigs play during Play sessions. Results showed a significant difference in tail movement duration between sessions ($DF = 15$; $t = -3.40$; $p < 0.01$; Student *t*-test for paired sample) with longer durations during the Play session than during the Control session. Tail movement frequency also varied significantly between Play and Control sessions ($DF = 15$; $t = 2.96$; $p = .01$; Student *t*-test for paired samples) with significantly higher frequencies during the Control session. A significant difference between sessions was also observed for ear movement frequency ($DF = 15$; $t = 4.69$; $p < 0.01$; Student *t*-test for paired samples), which was significantly higher during the Control session. In addition, 69% of the pigs (11 of 16) performed displacement behaviours during the Control session, while none of the pigs displayed this behaviour during the Play session. Finally, during the Play session, a negative correlation was found between tail movement duration and tail movement frequency ($rho = -0.79$; $N = 16$; $p < 0.001$). In conclusion, tail and ear movement changes are linked to play behaviour: in a play situation, tail movement duration increases, and ear movement frequency and displacement behaviours decrease, compared to a control situation (without enrichment). These results could be useful for improving the analysis of emotions in pigs and assessing animal welfare.

1. Introduction

An accurate assessment of animal welfare should involve the analysis of positive emotions, which may be frequent, in order to confirm positive animal welfare (Boissy and Lee, 2014). Nevertheless, there is a lack of clear and feasible indicators of positive emotions in pigs (Duncan, 2005). The availability of behavioural postures analogous to facial expressions in humans could be extremely valuable for the evaluation of animal emotions (Boissy et al., 2011).

Play behaviour is a likely candidate for indicating positive affective states and positive emotions (Horback, 2014; Mellor et al., 2009). Play behaviour diminishes during negative experiences, such as pain, and some authors consider it to be an indicator of animal welfare (Brown et al., 2015; Mintline et al., 2013). It is a commonly observed,

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characteristic behaviour of young mammals (Bekoff and Byers, 1998).

Reimert et al. (2013) suggested that tail movement could serve as a behavioural indicator of positive emotions in pigs: both tail wagging and tail posture changes occurred more often during rewarding than aversive events. Other studies have also reported tail wagging in pigs to be related to positive situations such as social greeting (Kiley-Worthington, 1975; Terlouw and Porcher, 2005) and play (Newberry et al., 1988). Reefmann et al. (2009a,b) suggested that tail movements may be an indicator of positive emotions in other species, such as sheep. Recently, other studies in mini-pigs showed a positive correlation between object play frequency and tail movement duration, as well as a positive correlation between social play duration and tail movement duration, in a group of pigs which had the possibility to play for ten minutes (Marcet Rius et al., 2018b).

While tail movements seem to be indicative of positive emotions, ear movements (i.e. changes between the ear postures ‘front’ and ‘back’) may indicate negative emotions in pigs (Reimert et al., 2013). Flattened ears have also been associated with negative situations in other species, namely sheep (Reefmann et al., 2009a,b), dogs (Kiley-Worthington, 1975) and horses (Freymond et al., 2014).

Currently, many indicators of poor welfare are used in animal welfare assessments. In our study, we decided to examine the presence or absence of two of these indicators, and to see if they would appear separately or concurrently with tail movement and/or ear movements. These findings would provide more information about the suitability of using tail and ear movements as indicators of positive, less positive or negative emotions in pigs. A high rate of agonistic behaviour or negative social behaviour could be considered as an indicator of poor welfare (EFSA, 2012; Temple et al., 2011). Displacement behaviours, such as continuous biting or licking of the wall, also appear to be of interest in welfare assessment. This otherwise normal behaviour is presented at an inappropriate time, appearing out of context for the situation, often as a consequence of frustration or conflict. Its presence is often linked to difficulties in coping with the environment. Displacement behaviours may also be observed in situations of arousal when there is no appropriate outlet for de-arousal (Landsberg et al., 2013).

The aim of the present study was to create a positive situation for the pigs, a play situation, in order to increase the probability of identifying potential indicators of positive emotions, thereby improving the assessment of welfare and emotions in pigs. The study first investigated whether the opportunity for play through the presence of toys was associated with an increase in tail movement and a decrease in ear movements. It is generally assumed that play behaviour in animals triggers positive emotions, and thus may trigger modifications in tail and ear movements, which could be used as behavioural indicators of emotions. An increase in tail movement could be an indicator of positive emotions, while a decrease in ear movement in a play situation compared to a control situation (without any stimulus) may mean that this intrinsic response is linked to a decrease in positive emotions that indicates a less positive (but not necessarily negative) situation. Both of these indicators could be very useful to better understanding emotions in pigs, an essential element to improving welfare assessment. Moreover, this study analysed the differences between other indicators of animal welfare, namely poor welfare, such as the presence or absence of displacement behaviours and of agonistic behaviour, in animals that were given the opportunity to play compared with those who could not, as well as the correlations between all these parameters.

2. Material and methods

The housing, husbandry and use of the animals involved in this experiment were carried out according to French and European legislation and in respect of the principles of replacement, reduction and refinement. The project, including this experimental procedure, was approved by the IRSEA's (Research Institute in Semiochemistry and Applied Ethology) Ethics Committee (C2EA125) and the French Ministry of Research (AFCE_201609_01).

2.1. Animals and housing

The mini-pigs (*Sus scrofa domesticus*) (n = 16: CM = 8; F = 8) involved in the study were a new strain resulting from cross-breeding of miniature breeds (Asian potbelly breeds: Vietnamese and Chinese) with conventional white hair breeds (Landrace and Large White), born and reared at the centre Specipig for breeding and biomedical research, in Barcelona, Spain. The pigs were entered into this study at the age of one year and had previously been involved in other studies (which consisted of measuring some physiological indicators using blood sampling in two different situations: with or without the presence of different types of enrichment material) (Marcet Rius et al., 2018a,b). Pigs were socialised to humans and had a positive human-animal relationship. Two individuals of the same sex and age were housed in each pen: 2.5 m^2 (1.85 m x 1.35 m), walls and doors of 0.90 m of high, slatted floor, presence of a feeder and a drinker in each pen. Groups were created after weaning to avoid fighting, so pigs were used to being together. They were housed in a controlled system in an experimental building designed for research, in two identical rooms (30 m^2), with monitored environment parameters: mean ambient temperature of 22 °C, same ventilation by 2 artificial ventilators in each room in the same position and 60% humidity. Pens were cleaned daily. Lights were on from 8.00 a.m. until 6.00 p.m. Pigs were fed twice a day with a special diet for mini-pigs maintained in restricted conditions for long-term trials (Special Diets Services, France) and had continuous access to drinking water.

2.2. Procedure

As mini-pigs were divided into two identical rooms of 8 individuals each, the study was organised over 4 consecutive days, with the animals from one room participating each day. Thus, each animal participated in the study for a total of two days in two different situations: one Control session and one Play session. Each animal was its own control, with each animal participating in both conditions. All pigs first participated in the Control session followed two days later by the Play session. Pigs were not moved to other rooms or pens to participate in the study, but they stayed in their own rooms and pens with their mates (in pairs). During Play sessions, pigs were placed in their own pens (2.5 m^2 in slatted floor), where two medium-sized dog toys (balls with attached ropes, Denta Fun, 7 cm/50 cm, 132 g, MonAnimalerie.net) were introduced. The test started when one operator put the toys on the floor in the centre of the pen and then exited the pen, while the pigs were present in the same pen. In each pen, two toys were provided to reduce conflict due to competition. During Control sessions, animals were also placed in their own pens and no toy was provided to them, as in their typical housing situation, with no extra stimulus. The behaviours of each pig were recorded during Play sessions and during Control sessions. Behaviours were scored from video using continuous recording with an ethogram (Table 1) and the help of Excel matrices (where frequency and duration of each behaviour could be measured thanks to two different lectures per video and per person), by two independent observers. Video recordings were also used to confirm that pigs played during Play sessions (Table 2). Prior to each session, faeces and urine were removed from the pen.

2.3. Statistical analysis

Data analysis was carried out using SAS 9.4 software Copyright (c) 2002–2012 by SAS Institute Inc., Cary, NC, USA. The significance threshold was classically fixed at 5%.

Reliability between the two observers who carried out the reading of the videos was calculated using Spearman’s or Pearson’s Correlation Coefficients using the CORR procedure depending on the normality of

Table 1
Ethogram used for video analysis of Play and Control sessions.

Behaviour	Definition
Object play/interaction with toy	Animal manipulates the toy or securely holds it in its mouth, energetically shaking it or carrying it around the pen (Newberry et al., 1988).
Social play	Two individuals interact in synchronisation, playing together with or without the toy, pushing the opponent with the head or shoulder or nudging (gentle snout contact) (Chaloupková et al., 2007).
Tail movements	Tail swinging in any direction, but mostly from side to side, so lateral tail movements (Kiley-Worthington, 1975; Reimert et al., 2013).
Ear movements	Any ear movement or ear posture change, including one or two ears (i.e. changes between the ear postures 'front' and 'back') (Reefmann et al., 2009a; Reimert et al., 2013).
Agonistic behaviour for competition	Actively displacing another pig, ramming or pushing another pig with the head with or without biting, aggressively biting any part of another pig or actively pursuing another pig (Chaloupková et al., 2007).
Displacement behaviour	A normal behaviour shown at an inappropriate time, appearing out of context for the situation, often as a result of frustration or conflict. It may also be observed in situations of arousal when there is no appropriate outlet for de-arousal (Landsberg et al., 2013). Examples in pigs: continuous biting or licking of the wall, excessive drinking, repetitive mastication with excessive salivation.

The frequencies and durations of each type of behaviour were analysed, except for displacement behaviour, where it was only noted the presence or absence. Behaviours could be overlapped, not mutually exclusive.

Table 2
Descriptive data of 600 s Play and Control sessions.

Pig	Session	OPF	OPD	SPF	SPD
1	Play	11	600	11	112
2	Play	10	600	13	129
3	Play	9	584	5	85
4	Play	6	599	5	97
5	Play	6	598	5	47
6	Play	6	600	5	59
7	Play	7	543	10	238
8	Play	8	579	7	251
9	Play	8	600	6	166
10	Play	8	600	7	135
11	Play	5	600	4	123
12	Play	6	589	5	136
13	Play	4	371	4	59
14	Play	7	600	2	51
15	Play	8	600	7	369
16	Play	6	244	7	431

OPF: Object play frequency
OPD: Object play duration
SPF: Social play frequency
SPD: Social play duration
There was no object play in Control sessions, as no object (toy) was provided.
There was no social play during the Control sessions.

data (normality was verified using the UNIVARIATE procedure): Spearman's Correlation Coefficient was used when normality was not verified for at least one variable, and Pearson's Correlation Coefficient, when normality was verified for both variables. This methodology was selected according to Martin and Bateson (2007).

For tail movement duration, tail movement frequency and ear movement frequency, normality was verified on the difference of Control session and Play session using the UNIVARIATE procedure. Comparisons between Control and Play sessions were performed using Student *t*-test for paired samples with the UNIVARIATE procedure.

Agonistic behaviour and displacement behaviours were transformed into binary variables and analysed using the McNemar's test using the FREQ procedure.

Correlations between all parameters (except for the binary variables) in each session were analysed using Spearman's or Pearson's Correlation Coefficients using the CORR procedure depending on the normality of data (normality was verified using the UNIVARIATE procedure), according to Martin and Bateson (2007): Spearman's Correlation Coefficient was used when normality was not verified for at least one variable, and Pearson's Correlation Coefficients, when normality was verified for both variables.

3. Results

3.1. Interobserver reliability

The reliability between the two observers carrying out the video analysis was calculated using Spearman's or Pearson Correlation Coefficients (Table 3). Reliability (inter-observer agreement) was considered high for all the parameters.

3.2. Comparisons between play and control sessions

Comparisons between Play and Control sessions were analysed for all parameters. Video recording confirmed that pigs performed play behaviour during all Play sessions and that they did not perform play behaviour (neither object nor social) during Control sessions (Table 2).

3.2.1. Tail movement duration

A significant difference was observed between sessions (DF = 15; $t = -3.40$; $p < 0.01$). More precisely, during Play sessions, tail movement duration was significantly higher than in Control sessions (Table 4).

3.2.2. Tail movement frequency

A significant difference was observed between sessions (DF = 15; $t = 2.96$; $p < 0.01$). In Play sessions, tail movement frequency was significantly lower than during Control sessions (Table 4).

3.2.3. Ear movement frequency

A significant difference was observed between sessions (DF = 15; $t = 4.69$; $p < 0.01$). Ear movement frequency was significantly lower during Play sessions than Control sessions (Table 4).

3.2.4. Agonistic behaviour

There was no significant difference between sessions regarding agonistic behaviour (DF = 1; S = 0.11; p = .74; McNemar's test).

Table 3
Interobserver reliability between the two observers carrying out the video analysis.

Parameter	Correlation Coefficient	P-value
Object play duration	Spearman: rho = 0.90	< 0.0001
Object play frequency	Spearman: rho = 0.90	< 0.0001
Social play duration	Spearman: rho = 0.97	< 0.0001
Social play frequency	Spearman: rho = 0.93	< 0.0001
Tail movement duration	Pearson: r = 0.90	< 0.0001
Tail movement frequency	Pearson: r = 0.85	< 0.0001
Ear movement frequency	Spearman: rho = 0.94	< 0.0001

Table 4
Tail movement duration (total of 600 s), tail movement frequency and ear movement frequency: comparison between Play and Control sessions.

Parameter	Session	N	Mean	Std Dev	Median	Minimum	Maximum
Tail movement duration	Control	16	331.84	161.81	362.25	71.00	528.50
	Play	16	445.47	136.76	501.75	200.00	594.50
Tail movement frequency	Control	16	20.28	8.66	21.50	7.00	37.50
	Play	16	13.97	7.69	12.75	1.50	28.00
Ear movement frequency	C$ontrol	16	10.03	6.95	9.25	0.50	23.00
	Play	16	2.66	2.26	2.00	0.00	6.50

3.2.5. *Displacement behaviours*

As no animal performed this behaviour during Play sessions, no statistical test could be executed, but descriptive data was computed. 69% of the pigs (11 of 16) performed displacement behaviours during the Control session. Some examples of these behaviours included: continuous licking or biting of the wall or floor for several minutes as well as standing or sitting with repetitive mastication and excessive salivation.

3.3. *Correlations between all parameters in play session*

During the Play session, a negative correlation was found between tail movement duration and tail movement frequency (rho = −0.79). According to Martin and Bateson (2007), r = 0.4–0.7 is considered to be a moderate correlation (substantial relationship), r = 0.7–0.9 represents a high correlation (marked relationship) and r = 0.9–1.0 represents a very high correlation (very dependable relationship). No other relevant association was found for the other parameters (no other correlations with, at least, r > 0.4).

4. Discussion

The main aim of this study was to investigate whether toy provision in pigs for the purpose of soliciting play behaviour was associated with tail movement and ear movement changes. As play behaviour occurs within a positive situation for the pigs, creating a play situation could increase the probability of identifying potential indicators of positive emotions. We first hypothesised that in a play situation, tail movement duration would increase, because, according to the literature (Reefmann et al., 2009a,b; Reimert et al., 2013), it appears to be linked to positive emotions. The second hypothesis was that in a play situation, with a positive stimulus, ear movement frequency would be lower than in a control situation, without any stimulus, because the literature (Reimert et al., 2013) suggests that it could be linked to negative emotions.

In our study, we did not create a negative situation, but a less positive situation, a control situation (with no extra stimulus). We thus hypothesized that there would be less ear movement in a play situation than a control situation.

We first confirmed by video analysis that pigs performed play behaviour after toy provision and that they did not perform play behaviour in control sessions. We subsequently found that tail movement duration was significantly higher during Play sessions, while ear movement frequency was significantly lower. Thus, our two main hypotheses were confirmed. Another interesting result was obtained regarding tail movement frequency, being lower during Play sessions than Control sessions. Finally, a negative correlation was found regarding tail movement frequency and tail movement duration.

Regarding tail movement duration in pigs, it was found that it was significantly higher in Play sessions than in Control sessions. This means that when pigs play, a behaviour which is believed to trigger positive emotions (Mellor et al., 2009), tail movement duration increases. This result suggests that the duration of tail movement behaviour over a short time period could be an indicator of positive emotions in pigs. Other authors have hypothesised that tail movement could be linked to positive emotions in some species (Kiley-Worthington, 1975; Reefmann et al., 2009a; Terlouw and Porcher, 2005) as well as in pigs (Reimert et al., 2013). Our results shed more light on the topic, supporting and confirming their hypothesis while adding new information and potential tools, such as a precise measure (duration of tail movement in a representative number of animals for a short period of time, e.g. 600 s). One of our previous studies (Marcet Rius et al., 2018b) showed a positive correlation between tail movement duration and object play frequency as well as social play duration, which encouraged us to continue exploring this field. These data suggested that the more a pig plays, the more it moves its tail, but we needed further research to confirm it. The present study confirms that tail movement duration is linked to play behaviour, strongly suggesting that it may be an indicator of positive emotions in pigs.

Our results showed that tail movement frequency was significantly lower in Play sessions than in Control sessions. In addition, analysis showed a negative correlation between tail movement duration and tail movement frequency. These results suggest that the longer tail movement lasts, the less frequent it becomes, which seems logical, because if we measure movement over a specific period of time, in this case, 10 min, if a pig starts and stops moving its tail many times, the duration would be shorter than a pig that moves its tail continuously throughout the 10 min. We may also consider that a pig that continuously experiences positive emotions, producing a continuous tail movement, could be in a more positive state than a pig that experiences positive emotion on and off, triggering a cycle of tail movement followed by a static tail, etc. Nevertheless, it is also possible that only if the duration approaches or equals the total observation time-period, the frequency and duration may be negatively correlated, but only in that circumstances. Thus, further research would be needed to confirm if the only real indicator of positive emotions in pigs appears to be the long duration of tail movement over a determined period of time, or if it would also be the mean duration of tail movement periods, or other kind of relationship between tail movement duration and tail movement frequency.

Regarding ear movement frequency, our results showed that it was significantly lower in Play than in Control sessions. According to the literature mentioned above, it appears that ear movement frequency or ear posture changes tend to be linked to negative situations and are less present in positive situations. Given that the provision of toys leading to the appearance of play behaviour triggers positive emotion in pigs, our results confirm that as ear movement frequency increases, the animals experience fewer positive emotions. Thus, as ear movements decrease, the animals experience more positive emotions, indicating a positive state of welfare. This study adds new information about this potential indicator of emotions in pigs, demonstrating that pigs show fewer ear movements in a positive situation than in a control situation (with no stimulus or enrichment).

Measures of agonistic behaviour showed no differences between sessions. We decided to introduce this parameter to provide more information about the positive situation (Play session) and the control one (Control session). The lack of a significant difference could be explained by the reduced number of agonistic behaviour in that group of mini-pigs, which is stable with no mixing of animals and no need to compete for resources. This fact results in a low incidence of the behaviour in both situations: Play and Control sessions.

Regarding displacement behaviours, none were observed during the Play session, but 69% of the pigs performed them during Control sessions. This was an interesting observation, meaning that in "normal" or "real" conditions, that group of mini-pigs were not in a very positive state of welfare, because no stimulus or enrichment was provided, and it could trigger the appearance of these abnormal behaviours. Nevertheless, in Play sessions, animals had something to do, their environment was enriched, and the behaviour disappeared. Displacement activities tend to appear in situations of psychosocial stress in animals (Maestripieri et al., 1992), so the fact that no animal performed these behaviours in a play situation reinforces the idea that this situation generates positive welfare. Thus, this result also supports tail movement as an indicator of positive welfare and confirms that pigs were in a more positive state during Play rather than Control sessions.

It was also interesting to observe that social play appears to be dependent on the presence of objects, at least, in our test conditions, as no social play appeared in Control sessions, but appears in Play sessions. It thus may be interesting to study the potential influence of the presence of toys on social play, which seems to stimulate the appearance of this behaviour, to confirm what we observed in our study.

Many authors have highlighted the need for more research to identify indicators of emotional states (Boissy et al., 2007; Duncan, 2005; Mellor et al., 2009), and particularly of emotional valence, in order to better assess animal welfare (Freymond et al., 2014). Our results present potential behavioural indicators of positive and negative emotions in pigs, tail movement duration and ear movement frequency, which could be used to improve animal welfare assessment in their actual breeding systems. Further research would thus be required to test these indicators with domestic pigs in intensive breeding systems.

5. Conclusions

In conclusion, tail and ear movement changes are influenced by play behaviour, which triggers positive emotions. More precisely, tail movement duration increases in a positive situation, while ear movement frequency is higher under control conditions and lower under positive play-inducing conditions, as does displacement behaviour. Thus, our study consistently suggests that high-duration tail movement is an indicator of positive emotions and that high-frequency ear movement is an indicator of decreased positive emotions in pigs, and thus a decrease in their welfare. These results could play an important role in improving the analysis of different emotions in pigs, thereby improving the assessment of animal welfare. This could be very useful in domestic pig breeding conditions, because they could provide new indicators for assessing emotions and animal welfare in a feasible, simple and practical manner.

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Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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3.3. Discussion:

This study showed that play behaviour, which is supposed to trigger positive emotions, modifies the appearance of tail and ear movements, with clear significant results, consistently suggesting that these movements can be considered indicators of emotions in mini-pigs. More precisely, a high tail movement duration could be considered as an indicator of emotions with a positive outcome, and a high ear movement frequency, as an indicator of emotions with a negative outcome. Interest exists in obtaining more information from other contexts for more thorough exploration of these intrinsic responses.

CHAPTER 4: Effects of straw provision, as environmental enrichment, on behavioural indicators of welfare and emotions in pigs reared in an experimental system

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4.1. Introduction:

Consequent to obtaining the results from the three preceding studies, a group of controlled studies were performed on mini-pigs with a controlled situation consisting of straw provision; this study focused on the behavioural parameters, with analysis of being performed for both situations (toys provision and straw provision) regarding both type of indicators (physiological and behavioural). So, we organised a study similar to the preceding one, but this time, with straw, and we analysed some behavioural parameters and compared between the two situations of the animals: straw or control sessions. We include the potential indicators obtained in the preceding studies and others, to obtain all the possible information about the state of the animals.

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Effects of straw provision, as environmental enrichment, on behavioural indicators of welfare and emotions in pigs reared in an experimental system

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ABSTRACT

The present study aimed to investigate whether straw provision in pigs increases positive emotions, indicated by tail movement, and reduces poor welfare indicators (agonistic and displacement behaviours), including indicators of negative emotions (ear movement). Comparisons between Straw and Control sessions were analysed from video recording for all parameters. 15 mini-pigs participated in a three-week study. Depending on the week, animals were included in Straw or Control sessions. During Straw sessions, pigs were placed in their own pens where straw was introduced and continuously provided for one week. During Control sessions, pigs remained in their pens with no additional stimulus. Pig behaviours were video-recorded four times during each session and scored. Results showed that ear movement frequency was significantly lower in the Straw than in Control session ($p = 0.005$); agonistic behaviour frequency and duration were significantly lower in the Straw than in Control session ($p = 0.013$ and $p = 0.0004$, respectively), and displacement behaviour frequency and duration were significantly lower in the Straw than in Control session ($p < 0.001$ and $p = 0.01$, respectively). Results suggest that straw provision reduces poor welfare but does not modify indicators of positive emotions selected for this study (tail movement frequency and duration). Our study also provides information about potential indicators of welfare, and more precisely, about emotions, which could also be useful to improve animal welfare assessment in pigs, obtaining more information about feasible behavioural indicators which could show the emotional state of the animals.

1. Introduction

The provision of straw in pig production systems is widely presumed to be beneficial to animal welfare (EFSA, 2014). There is weak evidence that the use of concrete flooring rather than straw is a risk factor for increased overall morbidity and mortality in pigs (Tuyttens, 2005). Straw is the material that most reduces the occurrence of harmful redirected behaviours (Whittaker et al., 1999). It also reduces many other welfare problems (van de Weerd and Day, 2009), such as aggression, tail biting and stereotypies (Burbidge et al., 1994). Nevertheless, previous studies (Marcet-Rius et al., 2018a) suggest that providing straw in a pig experimental system does not modify putative physiological indicators of positive animal welfare, like oxytocin and serotonin: no significant difference was observed between a group of pigs with a continuous provision of straw and a control group, over time; it thus appears that straw, and the possibility to perform exploratory and rooting behaviour, does not have an impact on plasma oxytocin and serotonin.

The concept of animal welfare includes not only physical welfare but also mental welfare, meaning that emotions are an important component of welfare (Broom, 1991; Mellor, 2012). It seems interesting to further analyse the real emotional state of the pigs when they have the opportunity to perform exploratory behaviour thanks to straw provision. To do so, a correct animal welfare assessment should not only include negative indicators, but should also analyse the production of positive emotions, in order to confirm that animals are in a positive

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welfare state (Boissy and Lee, 2014). The availability of behavioural postures analogous to facial expressions in humans could be extremely valuable for the evaluation of animal emotions (Boissy et al., 2011). In addition, it would be useful to have feasible indicators of emotions in pig production systems to avoid invasive and difficult practices such as blood sampling. Some authors have suggested that tail movement could serve as a behavioural indicator of positive emotions in pigs: both tail wagging and tail posture changes occurred more often during rewarding than aversive events (Reimert et al., 2013, 2015). Reimert et al. (2017) also showed that naive pigs tended to wag their tails more after the positive as compared to the negative treatment of the treated pigs. Recently, we (Marcet-Rius et al., 2018b,c) shed more light on the topic, supporting this hypothesis and results while adding new information and potential tools, such as precise measurements, as well as confirming that a high tail movement duration is linked to play behaviour. As it is generally assumed that play behaviour in mammals triggers positive emotions (Horback, 2014; Mellor et al., 2009), these results strongly suggested that long-duration of tail movement may be an indicator of positive emotions in pigs.

Regarding possible and feasible indicators of negative emotions, or a decrease of positive emotions, some authors have suggested that ear movements (i.e. changes between the ear postures 'front' and 'back') seem to be a potential behavioural indicator in pigs (Reimert et al., 2013; Marcet-Rius et al., 2018c). Flattened ears have also been associated with negative situations in pigs (Reimert et al., 2013, 2015; Goumon and Spinka, 2016) and in other species, namely sheep (Reefmann et al., 2009a,b), dogs (Kiley-Worthington, 1975) and horses (Freymond et al., 2014). It thus appears useful to analyse the appearance of both types of indicators to better understand the emotions of the pigs in a particular context.

Many indicators of poor welfare are currently used in animal welfare assessments. To identify the cause of a specific welfare outcome, several indicators need to be used (Welfare Quality, 2009). In our study, we decided to observe the presence of two of them, agonistic behaviour and displacement activities, to see if they would be emitted separately or together with tail movement and/or ear movement. A high incidence of agonistic behaviour or negative social behaviour could be considered as an indicator of poor welfare (Temple et al., 2011). Displacement behaviour is another interesting phenomenon to observe in this context. Displacement behaviours are thought to occur in conflict situations or in situations in which an animal is prevented from attaining its goal, so it is frustrated (Tinbergen, 1952). Its appearance is linked to a difficulty of the animal in coping with the environment (Landsberg et al., 2013), hence, it could indicate an inadequate environment, and a general poor welfare.

The aim of the present study was to investigate whether straw provision in pigs increases positive welfare, decreases negative welfare, or both. It is now widely recognised that the absence of negative welfare does not necessarily mean a high level of welfare, and that the expression of positive emotions is needed to achieve positive welfare (Boissy et al., 2007). Therefore, we used (i) certain potential indicators of positive emotions, such as tail movement duration, which could also be considered as an indicator of positive welfare, as welfare implicates mental and physical welfare (ii); certain potential indicators of negative emotions, or a decrease in positive emotions, in this case ear movements frequency, and (iii) some indicators of poor welfare, in this case a high incidence of agonistic and displacement behaviours.

2. Material and methods

The housing, husbandry and use of the animals involved in this experiment were performed according to French and European legislation and in respect of the principles of replacement, reduction and refinement. The project, including this experimental procedure, was approved by the IRSEA's (Research Institute in Semiochemistry and Applied Ethology) Ethics Committee (C2EA125) and the French Ministry of Research (AFCE_201609_01).

2.1. Animals and housing

The mini-pigs (*Sus scrofa domesticus*) (n = 15: Castrated males = 8; females = 7) involved in the study were a new strain resulting from cross-breeding of miniature breeds (Asian potbelly breeds: Vietnamese and Chinese) with conventional white hair breeds (Landrace and Large White), born and reared at the Specipig centre for breeding and biomedical research, in Barcelona, Spain. The pigs were entered into the study at sixteen months of age and had previously been involved in other non-invasive studies. Animals were previously socialised to humans and had a positive human-animal relationship. Pigs were housed in a controlled system in an experimental building designed for research, in two identical rooms (30 m^2), with monitored environment parameters: mean ambient temperature of 22 °C, same ventilation by 2 artificial ventilators in each room in the same position and 60% humidity. In one room there were four castrated males and four females; in the other room there were four castrated males and three females. Two pigs of the same sex and age were housed in each pen (2.5 m^2). Groups (pairs) were created after weaning to avoid fighting, so pigs were used to being together. Not all the pigs could be housed on pairs during the test, due to the death of some individuals before the beginning of the study. Apart from that, they were all in the same conditions. Pens were cleaned daily. Pigs were fed twice a day (at 8.00 a.m. and at 3.00 p.m.) with a special diet for mini-pigs maintained in restricted conditions for long-term trials (Special Diets Services, France) and had continuous access to drinking water. Lights were on from 8.00 a.m. until 6.00 p.m.

2.2. Procedure

The duration of the procedure was three weeks. Depending on the week, the pigs of one or both rooms participated in the study. In the first week, only the animals from room 1 were involved, participating in the Control session. In the second week, all the animals were involved: those from room 1 participated in the Straw session, while those from room 2 participated in the Control session. Finally, in the third week, only the animals from room 2 were involved, participating in the Straw session. This organisation was chosen for practical reasons so that each animal participated in the study for a total of two weeks into two different situations. All pigs first participated in a Control session followed by a Straw session, with each session lasting one week. Animals thereby served as their own controls. During the experimental procedure, pigs were housed in pairs, always with the same individuals, as in their normal life. As groups were created after weaning, fifteen months before the study, pigs were used to be together, being stressed when separated. Thus, for welfare reasons, as well as to not influence the normal behaviour of the pigs and so the results, it was decided to perform the study with the pigs housed in pairs. During the Straw session, pigs were placed in their own pens (2.5 m^2 in concrete floor), where 5 kg of straw were introduced in the floor of each pen (Straw from Coustenoble, 1 bd DEWAVRIN- BP 60,044 – 62,260 - Auchel, France). Every morning, the animal-keepers removed the dirty straw and provided the same quantity to each pen, in order to always have the same amount of straw in each pen. Straw was continuously provided and renewed in that way throughout the week. During the Control session, animals were placed in their own pens, but no straw was provided; the pigs were in the normal situation in their pen, with no extra stimulus. Pigs were video-recorded four times during each session on day 1, 3, 5 and 7 of each week, for 30 min, always at the same time (from 10.30 a.m. to 11.00 a.m.). Behaviours were scored from videos using continuous recording with an ethogram (Table 1). The first 10 min were not analysed, as the presence of the operator entering the pen to switch the cameras on could influence the pigs' behaviour. The last 10 min were also not analysed, as when operators were putting the

Table 1
Ethogram used for video analysis of Straw and Control sessions.

Behaviour	Definition
Exploratory behaviour	It consists of rooting, chewing, sniffing and manipulating the available rooting material (Studnitz et al., 2007).
Tail movement	Tail swinging in any direction, but mostly from side to side, so lateral tail movements (Kiley-Worthington, 1975; Reimert et al., 2013).
Ear movements	Any ear movement or ear posture change, including one or two ears (i.e. changes between the ear postures 'front' and 'back') (Reefmann et al., 2009a; Reimert et al., 2013).
Agonistic behaviour for competition	Actively displacing another pig, ramming or pushing another pig with the head with or without biting, aggressively biting any part of another pig or actively pursuing another pig (Chaloupková et al., 2007).
Displacement behaviour	Behaviour patterns characterized by their apparent irrelevance to the situation in which they appear (Maestripieri et al., 1992), often as a result of frustration or conflict. It may also be observed in situations of arousal when there is no appropriate outlet for de-arousal (Landsberg et al., 2013). Displacement behaviour observed in pigs: for more than one-minute period, continuous biting or licking of one part of the wall and repetitive mastication with excessive salivation.

The frequencies and durations of each type of behaviour were analysed, except for ear movements, considered as "event" by Martin and Bateson (2007), where only frequencies were analysed.
Behaviours could be overlapped, not mutually exclusive.
Tail movement frequency means the number of times that a pig starts moving the tail from side to side on the two-minute period.
Tail movement duration is expressed in seconds and means that a pig is moving its tail, considering a new movement when it stops the movement for, at least, 2 s (Marcet-Rius et al., 2018b and 2018c).

Table 2
Tail movement frequency and duration and ear movement frequency: comparison between Straw and Control sessions, for a total of 2400 s (40 min).

	Session	*N*	Mean	Median	Std dev	Minimum	Maximum	*p*-value
TMF	Control	15	47	47	15	15	67	0.23
	Straw	15	40	38	16	17	66	
TMD (s)	Control	15	950	1000	446	123	1843	0.46
	Straw	15	850	785	514	83	1875	
EMF	Control	15	110	96	56	36	221	< 0.01**
	Straw	15	70	66	23	42	109	

TMD: tail movement duration; TMF: tail movement frequency; EMF: ear movement frequency.
** high significance.

security overalls on and preparing the material in the airlock in order to switch the cameras off, animals could hear them, so it could influence their behaviour. Therefore, a total of 40 min per pig were analysed, for each session, meaning a total of 80 min per pig for the totality of the study.

2.3. Statistical analysis

Data analysis was carried out using SAS 9.4 software Copyright (c) 2002–2012 by SAS Institute Inc., Cary, NC, USA. The significance threshold was classically fixed at 5%. For all variables, the sum of the 4 videos was computed. Pen was included in the model as a random factor, to take into account the possible pen effect.

For tail movement duration, tail movement frequency and ear movement frequency, normality was verified, and comparisons between Control session and Straw session were carried out using the MIXED procedure. For agonistic behaviour frequency, agonistic behaviour duration, displacement behaviour frequency and displacement behaviour duration, normality was not verified and data was modelled with a general mixed Poisson model using the GLIMMIX procedure. As all the data consisted of discrete variables (times, for the frequencies, and seconds, for durations), the Poisson distribution was adapted.

Correlations between all parameters in each session were analysed using Spearman's or Pearson's correlation coefficient with the CORR procedure depending of the normality of data: Spearman's correlation coefficient "rho" was used when normality was not verified for at least one variable, and Pearson's correlation coefficient "r", when normality was verified for both variables. According to Martin and Bateson (2007), r = 0.4–0.7 is considered to be a moderate correlation (substantial relationship), r = 0.7–0.9 is considered to be a high correlation (marked relationship) and r = 0.9–1.0 is considered to be a very high correlation (very dependable relationship).

3. Results

3.1. Comparisons between Straw and Control sessions

Comparisons between Straw and Control sessions were analysed for all parameters, except for exploratory behaviour with the rooting material, as the animals of the Control session did not have the material so the comparison between sessions was not possible. Even though, it was important to measure it as it confirmed that all the pigs performed exploratory behaviour when straw was provided (exploratory behaviour duration in Straw session - Mean = 2139 s; Median = 2340 s; Total of observation = 2400 s).

No significant difference was observed between sessions neither for tail movement frequency (df = 14; F value = 1.55; p = 0.23) nor for tail movement duration (df = 14; F value = 0.59; p = 0.46) (Table 2). Concerning ear movement frequency, a significant difference was observed between sessions (df = 14; F value = 11.31; $p < 0.01$): in the Straw session, ear movement frequency was significantly lower than in the Control session (Table 2).

Concerning the frequency of agonistic behaviour, a significant difference was observed between sessions (df = 11; F value = 8.70; p = 0.01): in the Straw session, agonistic behaviour frequency was significantly lower than in Control session (Table 3). Regarding the duration, a significant difference was observed between sessions (df = 11; F value = 25.07; $p < 0.001$): in the Straw session, agonistic behaviour duration was significantly lower than in the Control session (Table 3).

Regarding the frequency of displacement behaviour, a significant difference was observed between sessions (df = 14; F value = 34.24; $p < 0.001$): it was significantly lower in the Straw session than in the Control session (Table 3). About the duration, a significant difference was observed between sessions (df = 14; F value = 8.78; p = 0.01): it was significantly lower in the Straw session than in the Control session

Table 3
Agonistic and displacement behaviours frequency and duration: comparison between Straw and Control sessions, for a total of 2400 s (40 min).

	Session	*N* obs	Mean	Median	Std dev	Minimum	Maximum	*p*-value
ABF	Control	12	3	2	3	0	9	0.01**
	Straw	12	1	1	2	0	5	
ABD (s)	Control	12	7	4	8	0	20	< 0.001***
	Straw	12	2	2	3	0	7	
DBF	Control	15	10	8	5	5	25	< 0.001***
	Straw	15	1	0	2	0	8	
DBD (s)	Control	15	1773	1971	655	419	2378	0.01**
	Straw	15	59	0	176	0	686	

ABD: agonistic behaviour duration; ABF: agonistic behaviour frequency; DBD: displacement behaviour duration; DBF: displacement behaviour frequency.
** high significance.
*** very high significance.

(Table 3).

3.2. Correlations between all parameters in Straw and Control sessions

Concerning Straw session, some positive correlations were found between agonistic behaviour frequency and agonistic behaviour duration ($r = 0.94$; $p < 0.0001$), displacement behaviour frequency and displacement behaviour duration (rho = 0.99; $p < 0.0001$); some negative correlations were found between exploratory behaviour duration and displacement behaviour frequency (rho = −0.73; $p < 0.001$), exploratory behaviour duration and displacement behaviour duration (rho = −0.73; $p < 0.001$), displacement behaviour frequency and tail movement duration (rho = −0.74; $p < 0.01$) and displacement behaviour duration and tail movement duration (rho = −0.72; $p < 0.01$). Regarding Control session, a positive correlation was found between agonistic behaviour frequency and agonistic behaviour duration (rho = 0.86; $p < 0.001$).

4. Discussion

The aim of the present study was to investigate whether straw provision in pigs increases positive welfare, and precisely, positive emotions, or decreases negative welfare, including negative emotions, or both. The results of the video analysis confirmed that pigs performed exploratory behaviour after straw provision. We observed fewer indicators of poor welfare with the presence of straw, and we did not observe an increase of the indicator of positive emotions. More precisely, no significant difference was found between Control and Straw sessions regarding tail movement duration and frequency. Regarding ear movement frequency, a significant difference was found between sessions, being lower in the straw than in the Control session. Concerning agonistic behaviour frequency and duration, as well as displacement activities frequency and duration, all were significantly lower in the Straw session than in the Control session. Many interesting correlations were found regarding all these parameters in Straw session, providing more information about the relationship between these behaviours and intrinsic responses and their relationship with the expression of emotions.

The fact that ear movement frequency was significantly lower in the Straw than in the Control session suggests that straw provision reduces negative emotions in pigs. A previous study (Marcet-Rius et al., 2018c) showed that ear movement frequency was lower in a play situation compared to a control situation, in accordance with the present results for straw provision, as both situations are designed to enrich the pigs' environment. According to the literature (Reimert et al., 2013, 2015, 2017), it appears that ear movement (measured as ear posture changes) is more linked to negative situations, and is less present in positive situations, suggesting that it could be an indicator of negative emotions or a decrease in positive emotions. Besides, this study (Marcet-Rius et al., 2018c) adds further information about these relationships, demonstrating that pigs showed less ear movement in a positive situation than in a control situation (with no stimulus or enrichment), and the results of the present study are in agreement with them.

Regarding the other indicators of poor welfare, the results showed that both indicators of poor welfare (agonistic and displacements behaviours) were significantly reduced in the straw provision situation compared to control (with no stimulus). These results suggest consistently that the straw reduces the appearance of poor welfare indicators, as the same result was obtained for agonistic behaviour frequency and duration as well as for displacement behaviour frequency and duration; both were significantly lower in the Straw session. Hence, it suggests that straw provision reduces poor welfare states, as indicated by the literature (Burbidge et al., 1994; Tuyttens, 2005; van de Weerd and Day, 2009; Whittaker et al., 1999). Additionally, these results confirmed that the control situation produces poorer welfare or a decrease in welfare for the pigs compared to a straw provision environment, giving more light to ear movements as potential indicator of negative emotions (or a decrease of positive emotions), which was significantly higher in the Control session. Thus, this study also suggests very consistently that a high-frequency of ear movements in pigs could be considered as an indicator of negative emotions, and so, a poor welfare indicator, appearing at the same time and in the same context as these strongly validated indicators (Maestripieri et al., 1992; Protocol Welfare Quality, 2009; Temple et al., 2011).

The results for tail movement duration as an indicator of positive emotions showed no significant differences between Straw and Control sessions. No differences were found regarding tail movement frequency. Thus, these results suggested that, in our test conditions, straw provision did not induce positive emotions in pigs. Other studies have also shown that straw provision does not modify putative physiological indicators of positive welfare, like oxytocin and serotonin (Marcet-Rius et al., 2018a). Nevertheless, other studies have shown that tail movement duration increases in positive situations, as for instance, in a play situation (Marcet-Rius et al., 2018b,c), which is supposed to trigger positive emotions (Mellor et al., 2009). Besides, Bolhuis et al. (2005) suggested that the provision of straw induces positive emotions, as it induces play behaviour. It seems interesting to mention that, some authors (Fraser, 1975) showed that with the presence of straw, pigs tend to rest more than without the straw, a fact that was also observed, even if not scored, during the viewing of the video recordings. When pigs are resting, tail movements are absent (or imperceptible), so it could be that this intrinsic response of tail movement might be attenuated in the Straw session for this reason, with the result of no significant difference between sessions. Further research focalised on the activity budget of pigs in some specific contexts would be needed, taking the resting time into account and evaluating its putative influence on tail movement, as well as including other indicators of positive emotions.

These results suggest that straw provision reduces poor welfare, including negative emotions, but it does not increase the appearance of the positive emotion indicators selected for this study. Hence, we may conclude that straw provision increases welfare, because poor welfare indicators are decreased, in accordance with the literature (Andersen and Bøe, 1999; Smith et al., 1998; Whittaker et al., 1999), but that it does not increase positive emotions, at least, not according to the indicator chosen for this study.

Much information was obtained concerning the correlations in the Straw session, in accordance with the results already mentioned. Most interestingly, a negative correlation was found between exploratory behaviour duration and displacement behaviour frequency, as well as between exploratory behaviour duration and displacement behaviour duration, confirming that when animals perform exploratory behaviour with the straw, they do not perform displacement activities. In addition, a negative correlation was found between displacement behaviour frequency and tail movement duration as well as between displacement behaviour duration and tail movement duration. These last correlations seem to indicate that when animals are in a poor welfare situation, represented by displacement behaviours, they move their tails less, which is coherent with the other results obtained and supports the use of long-duration tail movement as an indicator of positive emotions and positive welfare (Marcet-Rius et al., 2018b,c).

This research shows that enriching the environment with straw reduces the poor welfare situation induced by a poor environment, thereby reducing the suffering state of the animals, but it does not appear to produce positive emotions or pleasure in the pigs, at least, regarding our selected indicator. These results suggest that the straw provision compensates for the animals' poor environment, which would allow the pigs to "function" in the current intensive systems, or cope with this stressful environment. Nevertheless, this study suggests that it does not lead them to experience positive emotions, which seems essential to a positive welfare state. Further research would be necessary to explore more thoroughly this hypothesis such as investigating it in an intensive pig production system.

5. Conclusions

In conclusion, the straw provision reduces poor welfare in pigs, but it does not seem to produce positive emotions, at least, concerning the chosen indicator and in our test conditions. More precisely, it reduces the appearance of ear movements that are associated with negative emotions, and reduces agonistic and displacement behaviours, which seems important in the current production systems. Additionally, results show interesting correlations between different behaviours considered as positive or negative for the animals. Overall, these results may be useful to better understand the welfare state of the pigs in this context. It may also improve the animal welfare assessment of pigs, providing more information about simple and feasible behavioural indicators which reflect the emotional state of the animals. Our results also give rise to an important question about current pig intensive production systems: to ensure animal welfare, as well as the performance of the pigs, is it necessary to induce positive emotions in them or is it sufficient to simply eliminate the situations inducing poor welfare? Future studies including physiological and zootechnical measures as well as the incidence of positive emotions in a pig intensive system could be helpful to further explore this promising field.

Animal welfare implications

The main implication of this study was to understand the welfare state of the pigs when straw is provided, as a model of environmental enrichment, to determine whether it only reduces poor welfare or also increases positive emotions and so positive welfare. It also provides additional information about feasible behavioural indicators of animal welfare, which could be useful in improving pig welfare assessments.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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4.3. Discussion:

In this work, we confirmed that the study of some behaviours linked to straw provision, which provides an opportunity for pigs to perform exploratory and rooting behaviour, is of interest. We showed the difference between the effect on the pigs of these two types of enrichment material: straw and toys. These two different contexts do not have the same physiological or behavioural effect in the mini-pigs, showing different outcomes. This enrichment study also opened an interesting discussion about the emotional state of the animals, their positive welfare, their absence or of poor welfare, opening interesting questions and opening the door to future research.

CHAPTER 5: Can environmental enrichment affect tail and ear movements in pigs, as potential indicators of emotions?

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5.1. Introduction:

After the four controlled studies with mini-pigs in an experimental setting, we wanted to explore whether the results obtained could be reproduced under farm conditions with domestic commercial pigs. To do that, we organised a study in an experimental farm with commercial pigs. The aim was to investigate the parameters of tail and ear movements, and more precisely, to see if pigs move their tails more and their ears less when they interact with the enrichment, while trying to create a sort of positive situation and a control situation. It was possible to meet this aim because of international cooperation with the IRTA (Institute of Agrifood Research and Technology) in Spain.

animals

MDPI

Article

Can environmental enrichment affect tail and ear movements in pigs, as potential indicators of emotions?

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Simple Summary: The assessment of animal welfare should involve physical and mental welfare. Nevertheless, there is a lack of feasible indicators of positive emotions in farm animals, which causes difficulties to obtain a complete analysis of welfare. To improve the quality of life of farm animals, it is necessary to be able to assess their welfare in a valid and feasible way. This study aimed to determine whether environmental enrichment, understood as positive for animal welfare, could have an influence on the tail movement and ear movement in fattening pigs. Therefore, these indicators could be used to assess emotions, with a positive or a negative valence respectively, as suggested by previous studies in mini-pigs and pigs. Results showed that tail movement could be a valid and feasible indicator of positive emotions, since pigs provided with enrichment were found to present a higher percentage of tail movements. With regards to ear movements, the study revealed a need for further investigations. This research could play an important role in improving the analysis of different emotions in pigs, thereby improving the assessment of animal welfare in pig breeding systems using valid, feasible and non-invasive indicators of emotions.

Abstract: The inclusion of emotions' indicators in farm monitoring methods can improve welfare assessments. Studies in controlled conditions suggested that the increase of tail movement could be an indicator of positive emotions in pigs, while others proposed that an increase in ear movements is linked to negative emotions. This study aimed to investigate these indicators in pig farm conditions to analyze their validity and the effect that enrichment material could have on welfare according to these indicators. Thirty-six pigs received enrichment materials. Behavioral observations were performed. Results showed that tail movement duration was significantly higher when pigs performed a "high-use" of the enrichment materials than when they performed "low-use" (P = 0.04). A positive correlation was found between tail movement frequency and duration (r

= 0.88; P = 0.02). Consequently, the increase of tail movement could be considered an indicator of positive emotions in pigs. Results also support enrichment material as having a positive effect on welfare. Regarding ear movements, no significant difference was found. Future studies should investigate it thoroughly, as results could be useful for improving the emotions' assessment in pigs for welfare monitoring. Moreover, tail movements could be considered when assessing the enrichment effectiveness to induce positive welfare.

Keywords: Animal welfare; Pig assessment; Positive emotions; Negative emotions; Enrichment material

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1. Introduction

The assessment of farm animal welfare requires a good understanding of the animals' affective experiences, including their emotions [1]. Emotions are transient reactions to short-term triggering events, and their continued occurrence can cause longer-lasting affective states, which represent good or poor welfare [2]. The inclusion of indicators of emotions in farm monitoring methods can improve welfare assessments beyond the traditional focus on the mere absence of disease and distress [3] and good feeding and housing practices [4].

Currently, animal welfare assessments of farm animals do not always include the assessment of emotions, either positive or negative. In addition, when they are included, they could be influenced by a subjective assessment of the auditor [5]. This is why more objective and feasible indicators of the emotions of farm animals could be very useful for providing new insights on both animal welfare assessments and our understanding of the real state of the welfare, either positive or negative, of farm animals [3].

Emotional experiences are valenced, being perceived as positive or negative, rewarding or punishing, pleasant or unpleasant. Emotional experiences also vary in reported activation or arousal [6]. Emotional arousal could be defined as an emotional activation, in which animals' bodies experience heightened physiological activity and extremes of emotion, being positive, such as excitement, or negative, such as anger [7].

Some authors [8,9] have suggested that an increase in tail movement in pigs could be related to positive emotions: both tail wagging and changes in tail posture occur more often during rewarding than during aversive events. Other studies have also reported that increased tail wagging in pigs is related to positive situations such as social greetings [10,11] and play [12]. Recent studies performed in controlled conditions with mini-pigs about physiological and behavioral indicators of welfare and emotions [13,14,15] have provided clear results: tail movement duration was found to be significantly higher when animals performed play behaviors when provided with enrichment materials (middle-size dog toys) than when animals did not play as they were not provided with enrichment materials. The fact that tail movement duration increases when pigs play, a behavior that is believed to trigger positive emotions [16,17], suggests that a long duration of tail movement over a short time period could be an indicator of positive emotions in pigs.

Concerning negative emotions, Reimert and colleagues [8] suggested that an increase in the frequency of ear posture changes tended to be linked to negative situations and were less frequent in positive situations. Regarding other species, Boissy and colleagues [18] found that sheep point their ears backward when they face unfamiliar and unpleasant or uncontrollable situations, which are likely to elicit fear; Reefmann et al. [19,20] have also associated ears pointing backward with negative situations in sheep, which has also been shown by Kiley-Worthington [10] in dogs and by Freymond et al. [21] in horses. Marcet-Rius et al. [15] showed that, in controlled conditions, ear movement frequency was significantly lower in a group of mini-pigs that were allowed to play with an object, which they did during the entire experimental period, than in a group that was not allowed to play with an object. Thus, this research added new information about this potential indicator of emotions in pigs by demonstrating that pigs showed fewer ear movements in the positive situation than in the control situation (with no stimulus or enrichment). Another study [22] performed in controlled conditions with mini-pigs showed that ear movement frequency and the frequency and duration of other known indicators of poor welfare (agonistic behavior and displacement behavior), were significantly lower in a group provided with straw than in a control group, which was not provided with any manipulable material or other enrichment. The provision of straw in pig production systems is widely presumed to be beneficial to animal welfare [23,24], and the fact that ear movement frequency was significantly lower in pigs provided with straw than during the control session, together with two other indicators of poor welfare, strongly suggests that a high frequency of ear movement is more linked to negative emotions or at least is more common in a poor environment.

Intensive production systems are often very barren with concrete (slatted) floors and no substrate in which the animals can root [24]. Such environments hamper the pigs to express some key behaviors such as exploration and foraging [24]. As a consequence, harmful and manipulative behaviors such as ear and tail-biting often occur at high frequencies [25]. Successful enrichment should decrease the incidence of abnormal patterns of behavior and increase the performance of behaviours such as exploration, foraging, play, and social interaction, which are within the range of the animal's, normal, species-specific behavior [26]. Besides, it could also improve the performance, by improving the feed conversion ratio (reducing it) for example [27]. The provision of appropriate environmental enrichment to pigs is mandatory by law in Europe [28]. Additionally, the European Union is increasing their pressure towards leaving pigs undocked. Therefore, the need for finding indicators of positive welfare which could support the use of enrichment material that reduces the incidence of tail-biting is important. Tail movement could be a practical tool for farmers to assess positive animal welfare.

Considering that an increase in tail movement and an increase in ear movements are respectively indicators of positive and negative emotions in mini-pigs in a controlled system, the aim of the present study was to investigate these potential indicators in pigs provided with enrichment materials at an experimental farm with conditions resembling those found on commercial farms. In this study, enrichment materials were provided to pigs to allow exploratory behavior, which is a very important behavior in these animals [29]. We measured how the interaction with enrichment material could influence these potential indicators of emotions in pigs.

2. Materials and Methods

The housing, husbandry and use of the animals for the procedures described in this manuscript were carried out according to Spanish and European legislation. The project, including this experimental procedure, was approved by IRTA's (Institute of Agrifood Research and Technology) Ethics Committee.

2.1. Animals and housing:

The pigs (*Sus scrofa domesticus*) (n = 36; entire males) involved in this study came from a conventional commercial cross between (Landrace x Large White) dams with a Pietrain sire. All the pigs came from the same farm (Batallé® selecció), and they arrived at the experimental farm at IRTA at two months of age. All the pigs were tail-docked: the farm is on the process of implementing its action plan after carrying out a risk analysis of tail biting as required by EU recommendation 2016 and start to leave undocked pigs when action plan proves to work with docked pigs. They entered into the present study at 2.5 months of age. Thirty-six pigs were involved in the study and were divided into two groups living in identical rooms, with 18 pigs per room. In each room, there were three identical pens with six animals in each pen, with a stocking density of 0.9 m^2/pig. In every pen, each pig had a different color (blue, yellow, red, orange, green and white) tag to differentiate it from other pigs. Three different types of enrichment materials were constantly provided to the animals for three months, once for each pen of each room: straw in a rack, wooden logs and chains. For this experimental procedure, the important analysis was the interaction, or lack of interaction, with the enrichment regardless of the type as a way to create a positive situation for the animals and a control situation (lack of interaction). All the animals were provided with enrichment materials, meaning that all of them could interact with the materials and no animals were in a non-enriched pen. All the pigs were kept under the same housing conditions and managed in the same way and by the same stockpersons. The rooms where the pigs were housed and where the experiment was carried out had an automatic control system for regulating temperature and ventilation to maintain temperatures at 22±5°C. The pens had total slatted floor and bowl-type drinkers. Pigs were fed ad libitum with a commercial pig diet and had continuous access to drinking water. The pigs finished the present study at 5.5 months of age, which was the end of the fattening period, and were then kept at the farm for another experimental procedure to evaluate effectiveness of enrichment materials on pigs' responses to the novelty of transport.

2.2. Procedure:

At 2.5 months of age, the 36 pigs received environmental enrichment materials. Twelve pigs had a type of enrichment material: straw in a rack, wooden logs or chains. Straw was continuously provided in a rack and not on the floor to avoid large amounts of straw going underneath the slat system and to reduce cost for the farmers. Two wooden logs were hung perpendicularly from a chain in each pen, one on the door and another beside the door. Wooden-logs were near the floor but not touching it. Two chains were also attached perpendicularly to the metal fence bars of each pen, one on the door and another beside the door, and as with the wooden logs, they were near the floor but not touching it. Over three months, behavioral observations were performed once a week by one observer and with the help of one technician: the observer indicated the beginning and ending of each behavior (tail movement and ear movements), and the technician noted the frequencies and durations of each behavior with the help of a stopwatch on a data collection sheet. The interaction with the enrichment materials was scored as "yes" or "no" for each individual, during the two-minutes observation. More specifically, focal samplings of each pen were performed every Wednesday morning for 12 weeks, from 9 a.m. to 1 p.m., using a direct observation data collection sheet and an ethogram (Table 1). Each animal was observed for two minutes. Before starting the observations, the operators waited 2 minutes in front of each pen to allow the animals to acclimate to their presence minimizing any influence on their behavior.

Table 1. Ethogram used for behavioural observations of pigs by focal sampling.

Behavior	Definition
Tail movement	Tail swinging in any direction, but mostly from side to side, so lateral tail movements [9,10].
Ear movement frequency	Any ear movement or ear posture change, including one or two ears (i.e. changes between the ear postures 'front' and 'back') [9,15,19].
Interaction with the enrichment	Any manipulation, exploration and contact with the enrichment material.

The frequencies and durations of tail movement were analyzed.
Tail movement frequency means the number of times that a pig starts moving the tail from side to side on the two-minutes period.
Tail movement duration is expressed in percentage and means that a pig is moving its tail, considering a new movement when it stops the movement for, at least, two seconds [14,15].
Behaviors could be overlapped, not mutually exclusive.

2.3. Statistical analysis:

Data analysis was performed using SAS 9.4 software Copyright (c) 2002-2012 by SAS Institute Inc., Cary, NC, USA. The significance threshold was fixed at 5%. The experimental unit was the pen. First, differences between the 3 types of enrichment materials in relation to the 3 variables (tail movement frequency and duration and ear movement frequency) were analyzed over all twelve weeks. Before analysis, normality and homogeneity of variances were verified with the UNIVARIATE procedure and the GLM procedure, respectively, (using the HOVTEST = LEVENE option in the MEANS statement). As these conditions were met, a General Linear Mixed Model (including the room as a random effect) was carried out using the MIXED PROCEDURE., with multiple comparisons being performed using the LSMEANS statement in the MIXED procedure with the option ADJUST = TUKEY.

The second part of the statistical analysis consisted in the comparison of the variables (tail movement frequency, tail movement duration and ear movement frequency) with over all the twelve weeks in relation to the use of enrichment regardless of type, which was scored as "high" or "low": a high use of enrichment was considered when three or more animals of the pen interacted with the enrichment material, for a total of six animals in the pen, while a low use was when less than three pigs interacted with it. Before analysis, normality and homogeneity of variances were verified with the UNIVARIATE procedure and the GLM procedure, respectively, (using the HOVTEST = LEVENE option in the MEANS statement). As these conditions were met, a General Linear Mixed Model (including the room as a random effect) was carried out using the MIXED PROCEDURE, with multiple comparisons being performed using the LSMEANS statement in the MIXED procedure with the option ADJUST = TUKEY.

The third part of the analysis consisted in the correlations between the three variables to understand their potential relationships. As normality was verified (UNIVARIATE procedure), correlations between the three variables (tail movement frequency, tail movement duration and ear movement frequency) for the sum of the twelve weeks were assessed using Pearson's correlation coefficient using the CORR procedure. According to Martin and Bateson [30], r = 0.4-0.7 is considered to indicate a moderate correlation (substantial relationship), r = 0.7-0.9, a high correlation (marked relationship) and r = 0.9-1.0, a very high correlation (very dependable relationship).

3. Results

3.1. Comparison of the variables (tail movement frequency, tail movement duration and ear movement frequency) over all twelve weeks in relation to the type of enrichment material:

The main aim of this study was to create a positive situation for the pigs by providing enrichment materials and to compare if those potentially positive effects differed according to the influence of each type of material on the three behavioral variables. No significant differences were found between the three types of enrichment materials with regard to tail movement frequency (mean values for enrichment overall the 12 weeks: straw-rack = 2.19; wooden logs = 2.25; chains = 2.08; df = 2; F = 0.33; P = 0.72), tail movement duration (%) (mean values for enrichment overall the 12 weeks: straw-rack = 30.67; wooden logs = 28.21; chains = 27.95; df = 2; F = 0.36; P = 0.70) or ear movement frequency (mean values for enrichment overall the 12 weeks: straw-rack = 1.29; wooden logs = 1.38; chains = 1.40; df = 2; F = 0.11; P = 0.90) (Table 2). Therefore, the type of enrichment was not considered in subsequent analyses, and we focused only on whether enrichment materials were being used by the pigs.

Table 2. Comparison of the variables (tail movement frequency, tail movement duration and ear movement frequency) over all twelve weeks in relation to the type of enrichment material.

Tail movement frequency and duration and ear movement frequency							
		Type of enrichment					
Variable		Straw-rack	Wooden logs	Chains	df	F	P-value
TMF	N	24	24	24	2	0.33	0.72
	Minimum	0.8	0.3	0.0			
	Maximum	4.0	3.8	3.7			
	Mean	2.3	2.3	2.1			
	SE	0.2	0.2	0.2			
TMD (%)	N	24	24	24	2	0.36	0.70
	Minimum	3.2	1.5	0.0			
	Maximum	58.5	52.9	59.9			
	Mean	30.7	28.2	28.0			
	SE	2.9	2.5	3.0			
EMF	N	24	24	24	2	0.11	0.90
	Minimum	0.0	0.0	0.5			
	Maximum	3.3	4.0	3.8			
	Mean	1.3	1.4	1.4			
	SE	0.2	0.2	0.2			

TMF: Tail movement frequency
TMD: Tail movement duration (%)
EMF: Ear movement frequency
N: Number of pens (2 pens of each type of enrichment for 12 weeks)
SE: Standard Error

3.2. Comparison of the variables (tail movement frequency, tail movement duration and ear movement frequency) over all the twelve weeks in relation to the use of enrichment (scored as "high" or "low") regardless of type:

A statistical trend was found between the pens with a low or high use of enrichment material for tail movement frequency, which was higher for a high use of enrichment (mean for high use = 2.50; mean for low use = 1.89; df = 1; F = 3.76; P = 0.06). A significant difference was found for tail movement duration (%) being higher for the high use of enrichment (mean for high use = 33.16; mean for low use = 25.17; df = 1; F = 4.88; P = 0.04). No significant difference was found for ear movement frequency (mean for high use = 1.25; mean for low use = 1.45; df = 1; F = 0.28; P = 0.60) (Table 3).

Table 3. Comparison of the variables (tail movement frequency, tail movement duration and ear movement frequency) over all the twelve weeks in relation to the use of enrichment (scored as "high" or "low") regardless of type.

Tail movement frequency and duration and ear movement frequency						
		Use EM				
Variable		High	Low	df	F	P-value
TMF	N	34	38	1	3.76	0.06
	Minimum	0.5	0.0			
	Maximum	3.8	4.0			
	Median	2.7	1.8			
	SE	0.1	0.2			
TMD (%)	N	34	38	1	4.88	0.04*
	Minimum	1.5	0.0			
	Maximum	59.9	54.4			
	Median	32.4	25.3			
	SE	13.4	12.6			
EMF	N	34	38	1	0.28	0.60
	Minimum	0.0	0.0			
	Maximum	4.0	3.3			
	Median	1.1	1.3			
	SE	0.2	0.1			

Use EM: Use of enrichment material.
High use of enrichment: when 3 or more pigs in a pen interact with the enrichment material.
Low use of enrichment: when less than 3 pigs in a pen interact with the enrichment material.
TMF: Tail movement frequency.
TMD: Tail movement duration (%).
EMF: Ear movement frequency.
N: Number of pens (2 pens of each type of enrichment for 12 weeks).

3.3. Correlations between the three variables (tail movement frequency, tail movement duration and ear movement frequency) for data summed over the twelve weeks:

A positive correlation was found between tail movement frequency and tail movement duration ($r = 0.88$; $P = 0.02$). No other relevant correlations were found neither between tail movement frequency and ear movement frequency ($r = 0.42$; $P = 0.41$) nor between tail movement duration and ear movement frequency ($r = 0.03$; $P = 0.95$).

4. Discussion

The results showed that tail movement duration was significantly higher when the animals interacted more with the enrichment materials than when they did less during almost all the fattening period (twelve weeks). A statistical trend was also found for tail movement frequency, being also higher for the high use of enrichment. A positive correlation was found between tail movement duration and tail movement frequency. This suggests that a high tail movement duration could be an indicator of emotions in fattening pigs with a positive outcome, according to the literature [8,9,14,15,31]. Furthermore, it suggests that a high tail movement frequency seems to be linked to positive emotions, even if more studies would be needed to confirm it, taking into account different total durations of observation and perhaps the ratio between these two parameters. These results suggest that tail movement in pigs could be linked to the use of enrichment materials and therefore to exploratory behavior, which is very important in pigs. The tail movement could also be used to indicate positive emotions, which would indicate positive animal welfare.

Concerning the relationships between the three parameters (tail movement frequency, tail movement duration and ear movement frequency), the results showed a positive correlation between tail movement frequency and tail movement duration. This suggests that an increase of tail movement, either in frequency or duration, is linked to positive emotions and that both could be used as indicators of emotions with a positive outcome. Previous studies [15] suggested that, over a ten-minute observation period, the high tail movement duration could be a useful indicator, although the results for frequency were not significant. These results demonstrate that tail movement is a valid indicator to assess emotions. Besides, it could also be used as a potential tool to evaluate effectiveness of enrichment materials to prevent tail biting, among other animal welfare problems. Apart from the duration of tail movement during a set period of time, another feasible measure may be the mean duration of tail movement episodes expressed as a ratio of duration to frequency, but more research is necessary to confirm this.

Interestingly, this study also suggested that mini-pigs could be a suitable model of domestic commercial pigs, since results are in accordance with precedent studies with mini-pigs. There are some studies on behavior and welfare of different breeds of mini-pigs [32] and other studies suggesting that welfare indicators of commercial pigs can be used for mini-pigs [33]. Nevertheless, as far as we know, there were no studies until now showing that mini-pigs seem to be a suitable model of domestic commercial pigs.

It is important to remember that all the animals in this study had some type of enrichment materials, meaning that all of them could use these materials and that none of the animals was in a non-enriched pen. This represents a limitation in our study as we did not have a control group and could only compare pens between them when they use more or less the enrichment materials. Providing enrichment materials to all pigs is mandated by law [28], and many studies have already shown their benefits [24,29,34]. Therefore, in farm conditions, which were used in the present study, no breeders are supposed to house their pigs without enrichment materials. Nevertheless, the lack of a control group turned into a positive aspect, as even though all the animals had the possibility of using enrichment materials, and so were not in a very poor environment, they still showed more tail movement when they interacted with the enrichment materials at specific moments than when they did not. This suggests they were more aroused and with a positive valence [6] at that moment and that this triggered a higher expression of the response behavior (tail movement). In general, the results showed a longer tail movement duration when animals used the enrichment materials, or used them more frequently, than when they did not use them, or used them less frequently, which suggests that tail movement is linked to positive emotions and, therefore, to animal welfare. These results are in accordance with the findings of previous studies [9,10,15]. Previous work [14,15] has shown similar results in mini-pigs in an experimental system, and the present study shows that these results can be reproduced at large experimental farms with features very similar to those of commercial farms. These methods may be applied in the latter.

Another limitation was that the pigs were sometimes lying down or sitting during the observation period and while using the enrichment materials. In these cases, the tail movement could not always be measured because the tail could not be observed or it could not move due to direct contact with the floor, both resulting in the behavior being scored as no tail movement. This factor reduced the values for tail movement duration and frequency and could have directly affected the results. This drawback has also been observed by Reimert and colleagues [9]. One possible solution to reduce resting behaviors during observations in future studies could be having the observer enter the pen before performing the observations to make the pigs stand up, as was performed in the Welfare Quality Protocol [4].

Another interesting topic to explore thoroughly was the fact that all the pigs of the experiment were tail-docked. It would be interesting in the future to compare tail movement in pigs with and without tail-docking, as it is a practice which should be avoided, considering the current European legislation of 2008 [35]. Tail-docking may affect tail movements, as well as the communication between animals and social interactions [36]. Besides, European Union is pressuring to totally fulfil the legislation and a recommendation has been published in 2016 [37]. The use of valid indicators such as tail movements could help the farmers to prevent tail biting.

No significant difference was found for ear movement frequency in relation to whether enrichment materials were used for over all the twelve weeks. Previous studies suggested that ear posture changes in pigs could be linked to negative emotions [8]. Other studies have consistently suggested that a high ear movement frequency could be a direct indicator of negative emotions by showing that the frequency was significantly higher in barren conditions than in enriched conditions [14,15,22]. These last studies were performed in controlled conditions with mini-pigs, so the aim of the present study was to investigate whether these results could be reproduced in experimental farm conditions with domestic pigs with conditions that were very similar to those at commercial farms. We did not obtain the same results as those obtained with mini-pigs. One possible explanation could be the anatomic difference between the ears of mini-pigs (small in proportion of the head) and (Landrace x Large White) x Pietrain pigs (very large and heavy in proportion of the head). The auricles of pigs are mobile, and they can move for better detect and locate the sound [38]. The anatomical structural base of auricles as well as their form could vary depending on the breed of different domestic animals, which could also affect the movement of ears [38]. It could be possible that large and heavy ears are less mobile than small ones, as suggested by Wei et al. [39]. Another possible explanation could be that the rooms where the fattening pigs were housed were noisier than the rooms where mini-pigs were housed, and this could affect the ear movements. Finally, it could also be possible that the two-minutes observation per week was not enough to obtain some significant results, compared to the observation period in precedent studies [15,22]. Further research would be interesting to investigate these possible explanations as well as to do it in different breeds of fattening pigs with different type of ears.

5. Conclusions

In conclusion, this study supports the use of enrichment materials for pigs, suggesting that they increase the feeling of positive emotions. This study also provides new perspectives on the evaluation of emotions in farm animals and investigates the suitability of using a high tail movement frequency and duration as indicators of emotions in pigs with a positive outcome, although further research is needed to investigate this possibility thoroughly in real conditions. Besides, this indicator could be used as a practical tool for farmers. This study also investigated the use of a high ear movement frequency as a possible indicator of emotions with a negative outcome, which was shown in previous controlled studies. However, the present study, performed in conditions of an experimental farm, was not able to confirm this association. Nevertheless, interesting hypotheses are proposed, like the differences in the size of ears in proportion to the size of the head between mini-pigs and (Landrace x Large White) x Pietrain pigs which could lead to differences in ear mobility, among others. Therefore, future studies will be planned to continue investigations in this field of research, and the results may be very useful for improving animal welfare assessments of pigs in different breeding systems and to provide a better understanding of their emotions, as an important part of animal welfare.

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5.3. Discussion:

The results obtained encouraged us to continue in this field of research, suggesting that a high tail movement duration could also be an indicator of emotions in fattening pigs with a positive outcome. The finding provided new perspectives on how to evaluate the emotions in fattening pigs, including investigating the suitability of using a high tail movement frequency and duration, as well as, perhaps, the ratio of frequency and duration, as indicators of emotions to include it in the existent welfare assessments. Further research would thus be organised to investigate this possibility thoroughly.

GENERAL DISCUSSION

The present work mainly aimed to investigate some potential physiological and behavioural indicators of positive animal welfare. To do that, an integration of several measures, including potential indicators of positive emotions, negative emotions and poor welfare, were measured in a series of experiments. Four studies were carried out with mini-pigs, as a model of commercial pigs, in an experimental setting. One last study was performed in an experimental farm with commercial pigs, with conditions very similar to the intensive pig production system.

The first and second studies were more focused on the physiology, analysing some potential physiological indicators of animal welfare, i.e., peripheral oxytocin and peripheral serotonin, in a positive context for the pigs. The third and fourth studies, were more focused on the behaviour, analysing some potential indicators of welfare and emotions, such as tail and ear movements, among others, also creating a positive context for the pigs. The last study was performed under farm conditions with commercial pigs, with a high number of individuals, to investigate whether the same results obtained with mini-pigs could be reproduced under those conditions.

The first study entitled "*Providing straw to allow exploratory behaviour in a pig experimental system does not modify putative indicators of positive welfare: peripheral oxytocin and serotonin"* (see chapter 1) aimed to investigate the potential effect of straw provision on peripheral oxytocin and serotonin. Straw provision in pigs is widely presumed to be highly beneficial for their welfare (Burbidge et al. 1994; Studnitz et al. 2007; Tuyttens 2005; van de Weerd and Day 2009; Whittaker et al. 1999), and the authors wanted to produce a context linked with a high level of welfare in pigs, which would allow them to analyse some physiological effects. The results suggested that straw provision in pigs does not modify oxytocin or serotonin concentration in a controlled system, even if some difficulties have been found that could have influenced the results: the handling effect during blood sampling, the individual differences, and the duration of the study, among others.

The second study entitled *“Selection of putative indicators of positive emotions triggered by object and social play in mini-pigs”* (see chapter 2) was very similar to the first one, but this time, the positive context consisted of providing medium-sized dog toys as an enrichment material, to encourage play. Because play behaviour is considered to produce positive emotions (Mellor et al. 2009; Boissy et al. 2007; Brown et al. 2015; Donaldson et al. 2002; Held and Špinka 2011; Mintline et al. 2013), making the pigs play is supposed to produce positive emotions. Thus, this time the provision was different, as toys were provided at specific times during the day, whereas the straw was provided continuously. We obtained many interesting results: for those animals that played, as toys were provided to them, they showed more stable levels of oxytocin after playing and after blood sampling, suggesting that those pigs may already be in a balanced state due to the presence of environmental enrichment and the opportunity to play, unlike the pigs without enrichment; these pigs exhibited an increase in plasma oxytocin following two blood sampling events, which could activate a need to cope. This finding suggests that the pigs that are allowed to play are more capable of coping with stressful situations, and importantly, the latter part of that statement is the first definition of animal welfare. Regarding the long-term effect of toy provision on oxytocin, the results showed no effect, suggesting that the short duration and frequency of the stimulus (total of 30 minutes per day for 3 weeks) may not be sufficient to affect this neuromodulator, or that no evidence exists to show that object play, contrary to social play (or social interaction), could affect plasmatic oxytocin (Handlin et al. 2011;

Odendaal and Meintjes 2003). Concerning peripheral serotonin, no significant results were found, either in the first or in the second study, suggesting that peripheral serotonin is not linked to the provision of enrichment material in pigs, so it was excluded as a possible indicator of welfare or at least under our test conditions. In addition, some positive correlations were found concerning physiological and behavioural measures: a positive correlation with object play frequency and tail movement duration, as well as between social play duration and tail movement duration, suggesting that the more a pig plays, the more it moves its tail, giving rise to our interest on this potential behavioural indicator of positive emotions in pigs.

The third and the fourth study were more focused on behavioural parameters, as a second step to investigate some potential and feasible indicators of positive emotions and positive welfare. To do that, situations more similar than in the previous studies were created with enrichment material: the third study with toys and the fourth one with straw provision.

The study of the toys entitled *“Tail and ear movements as possible indicators of emotions in pigs”* (see chapter 3) showed that tail movement duration was significantly higher when pigs played than when they did not, whereas ear movement frequency was significantly lower. Nevertheless, tail movement frequency was significantly lower in play than in control sessions. As play behaviour is supposed to trigger positive emotions, these results suggest, according to the literature, that a high tail movement duration and a low ear movement frequency are linked to emotions, showing a positive state of welfare, being thus potential indicators of emotions in pigs. Regarding the results of the tail movement frequency, which was contrary to tail movement duration, could have been affected by the duration of the observation period. In addition, a negative correlation was found between the two variables. Possibly, the frequency and duration may be negatively correlated but only in those circumstances when the duration approaches or equals the total observation time. In fact, in the following study with different durations of observation, the results were different, showing significantly higher tail movement frequency when pigs interacted with the enrichment material than when they did not.

The fourth study entitled *“Effects of straw provision, as environmental enrichment, on behavioural indicators of welfare and emotions in pigs reared in an experimental system”* (see chapter 4) showed that indicators of poor welfare (agonistic behaviour, displacement behaviours and ear movement frequency) were decreased when straw was present compared to when absent, whereas the potential indicators of positive emotions (tail movement duration and frequency) were not increased. These results suggest that straw provision reduces negative emotions and negative welfare in pigs. Nevertheless, it does not seem to produce positive emotions, or at least, as shown by our selected potential indicator (tail movements). Our first study also suggested that straw provision does not modify putative physiological indicators, such as oxytocin and serotonin, of positive welfare. Another possibility could be that, as pigs rest more when straw is present than when it is not (Fraser 1975) and that when they rest, tail movement could not be scored, which may have influenced the results of this parameter; this fact has also been observed by Reimert and colleagues (2017).

The aim of the fifth study entitled *“Can environmental enrichment affect tail and ear movements in pigs, as potential indicators of emotions?”* (see chapter 5) was to create a positive situation for the pigs by providing enrichment materials, analysing the potential behavioural indicators of welfare: tail and ear movements. This time, the animals involved in the procedure were commercial pigs, under farm conditions, very similar to real commercial production conditions. The results showed that tail movement duration was significantly higher when the pigs interacted more with the enrichment than when they did less during all fattening period, suggesting that high tail movement duration could be an indicator of emotions in fattening pigs with a positive outcome, as found with mini-pigs. A statistical trend was found for tail movement frequency, being also higher for the high use of enrichment. Moreover, a positive correlation was found between tail movement frequency and tail movement duration, suggesting that tail movement in pigs could be linked to the use of enrichment materials and to exploratory behaviour, which is very important in pigs. Thus, it seems that tail movement could be used to indicate positive emotions, and positive animal welfare, not only in mini-pigs but also in commercial pigs. These results also underlined the importance of environmental enrichment in pigs, as it seems that it increases the feeling of positive emotions. Concerning ear movement frequency, no significant differences were found between the interaction or not with the enrichment, in contrast to the results obtained with mini-pigs under controlled conditions. One possible explanation could be the anatomic difference between the ears of mini-pigs (small in proportion of the head) and (Landrace x Large White) x Pietrain pigs (very large and heavy in proportion of the head). Another possible explanation could be that the rooms where the fattening pigs were housed were noisier than the rooms where mini-pigs were housed, and this could affect the ear movements. Finally, it could also be possible that the two-minutes observation per week was not enough to obtain some significant results, compared to the observation period in precedent studies. Research would be interesting to investigate these possible explanations as well as to do it in different breeds of fattening pigs with different type of ears.

These findings are important for the following reasons:

- They suggest improvements to animal welfare in production systems and its assessment, with new potential indicators of animal welfare.
- The indicators are feasible, which is essential under farm conditions.
- A better understanding of animal emotions is important to animal welfare.
- The link among the different fields of veterinary sciences—physiology, ethology, welfare, among others—to describe the state of animals in a specific context, serves as a model for connecting different sciences to obtain more information.
- A model of investigation has been created to obtain some potential indicators to assess emotions and welfare in different species.

These findings open some doors to improve the assessment of pig welfare, as well as to improve our understanding of their emotions and their real welfare state and quality of life. In addition, this work could play an important role in improving the analysis of different emotions in pigs and thus in other species. Further research would be required to learn more about these potential indicators of animal welfare and animal emotions under real farm conditions and their applications to improve animal management and care.

OVERALL CONCLUSIONS AND PERSPECTIVES

Overall, these experiments allowed us to accomplish the following:

1. Some physiological and behavioural indicators of positive welfare were investigated, as they are the two categories of indicators that are animal-based measures.

    a. The stability of peripheral oxytocin can be considered a potential measure of positive welfare, although further research is required.
    b. Peripheral serotonin does not seem to be a potential measure of welfare in pigs, but more research is needed to investigate it thoroughly.
    c. High tail movements in pigs, either in duration or frequency, could be an indicator of positive emotions in pigs and hence an indicator of positive welfare.
    d. High frequency of ear movements seems to be related to negative situations and the emission of negative emotions, although future research is needed to validate this.
    e. These potential physiological and behavioural indicators of emotions and welfare could be very useful for future applications in pig breeding systems to improve the assessment of animals, as well as their quality of life.

2. Attention was focused on emotions, which are an important part of animal welfare, although not usually included in animal welfare assessments; further research is needed on this subject:

a. A model was created to assess positive emotions in an experimental setting that allows pigs to play and/or interact with different types of enrichment material.
b. High tail movement frequency and duration and high ear movement frequency were identified as potential indicators of emotions in pigs, with positive and negative outcomes, respectively.
c. The behavioural indicators of emotions are feasible, practical, and objective, overcoming the main difficulties with existing indicators, making the current findings important.

3. Concerning the behavioural indicators, the feasibility was considered, as they are easy and practicable to be measured.

4. The study began under controlled conditions using the domestic pig (mini-pigs) as a model and concluded with studies on commercial pigs under farm conditions.

   a. Mini-pigs developed for research were used as a model for domestic pigs used in production, which was an innovative approach that we developped.
   b. The different studies demonstrated that mini-pigs were a suitable model of domestic commercial pigs.
   c. An important model of research, which could serve as an example for other research institutes, was used. This approach takes into account the 3R's of ethical principles (replacement, reduction and refinement),

while improving the quality of the work and the practicability of the operators working with the animals.

5. New perspectives were introduced to include new indicators of emotions and positive animal welfare in farm animal welfare assessments, to improve these indicators, and to prevent welfare problems on farms.

    a. This work opens the door for new measures to assess animal welfare, while also considering emotions, and combining different veterinary sciences that are complementary (ethology, physiology and welfare).
    b. This approach could serve as a model for assessing emotions and positive welfare in farm species and also in all type of animals kept by humans.

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ANNEX

1. European legislation regarding the protection of pigs during the production system

1.1. Most relevant points of the European directives

Four European directives exist for the protection of pigs, and one directive, for the protection of farm animals, including pigs. These directives are mandatory for all the Member States. Each Member State could choose to be stricter in the protection of their farm animals, but at the least, they must follow all the points of the last directive in force. The most important points of each directive are summarized in this chapter to show the minimal standards of welfare of the pig production system and its evolution.

1.1.1. Council Directive 91/630/EEC of 19 November 1991 laying down minimum standards for the protection of pigs

Articles:

- Council Directive 91/630/EEC establishes the floor area available for weaners and rearing pigs in groups, depending on their weight, and is not valid for holdings with fewer than six pigs.

Average weight, kg	m^2
10 kg	0.15
between 10 kg and 20 kg	0.20
between 20 kg and 30 kg	0.30
between 30 kg and 50 kg	0.40
between 50 kg and 85 kg	0.55
between 85 kg and 110 kg	0.65
more than 110 kg	1.00

- The construction or conversion of installations to tether sows and gilts is prohibited, except for holdings with fewer than five sows with their piglets.
- The Commission, with the advice of the Scientific Veterinary Committee, shall submit a report to the Council, considering the welfare, and especially the welfare of sows, which will be accompanied by some proposals to improve welfare.
- Inspections to ensure the accomplishment of the directive are mandatory and under the responsibility of the competent authority.
- Animals imported from non-member countries must receive treatment at least equivalent to animals of member countries and should be accompanied by a certificate.

Annex:

Chapter 1 - General conditions:

- Materials used for the construction of housing must be safe for the pigs and capable of being cleaned and disinfected.

- To avoid electric shocks, electrical circuits and equipment must be installed in accordance with national rules.
- Insulation, heating and ventilation of the building must ensure that air circulation, dust level, temperature, relative air humidity and gas concentrations are kept within limits that are not harmful to the pigs.
- All automated or mechanical equipment must be inspected at least once daily. Where defects are discovered, these must be rectified immediately or, if this is impossible, appropriate steps must be taken to safeguard the health and well-being of the pigs until the defect has been rectified.
- Natural or artificial lighting is mandatory: if artificial, it should be provided between 9 a.m. and 5 p.m. In addition, suitable lighting (fixed or portable) and strong enough to allow the pigs to be inspected at any time must be available.
- All pigs must be inspected by those responsible for the animals at least once per day. Any pig that appears ill or injured must be treated appropriately without delay. Sick or injured pigs must be isolated in adequate accommodation with dry, comfortable bedding. Veterinary advice must be obtained as soon as possible for pigs that are not responding to the stockperson's care.
- Measures to prevent fighting must be taken. More aggressive pigs or those suffering from the most aggression must be kept separate from the group.
- The accommodation for pigs must allow them to lie down, rest and stand up without difficulty, being clean and allowing them to see other pigs.
- Tethers are allowed, but they must not cause injury to the pigs. Each tether must be inspected regularly and adjusted, it needs to be of sufficient length to allow the pigs to move, avoiding any risk of strangulation or injury.

- Housing, pens, equipment and utensils used for pigs must be properly cleaned and disinfected. Faeces, urine and uneaten or spilt food must be removed as often as necessary.
- Floors must be smooth but not slippery. The lying area must be comfortable, clean and adequately drained. Where bedding is provided, this must be clean, dry and not harmful to the pigs.
- A diet appropriate to each age, weight, behaviour and physiological need is mandatory. All pigs must be fed at least once per day, and each pig must have access to the food at the same time as the others in the group.
- All pigs over two weeks of age must have access to a sufficient quantity of fresh water or be able to satisfy their fluid intake needs by drinking other liquids.
- All pigs must be able to obtain straw or any other suitable material or object to prevent tail-biting and to satisfy their behavioural needs.

Chapter 2 - Specific provisions for various categories of pigs:

Boars:

- Boar pens must allow them to turn around, hear, smell and see other pigs. The lying area must be dry and comfortable, with a minimum size of 6 m^2. A larger area must be provided when pens are used for service.

Sows and gilts:

- If necessary, pregnant sows and gilts must be treated against external and internal parasites.
- If sows and gilts are placed in farrowing crates, animals must be cleaned.
- If necessary, suitable nesting material should be provided.

- A clean, adequately drained and comfortable lying area must be provided.
- An area behind the sow or gilt must be available for the farrowing (natural or assisted).
- Farrowing crates must have some means of protecting the piglets.

Piglets:

- If necessary, piglets must be provided with a source of heat and a dry and comfortable lying area where all can rest at the same time.
- The castration of male pigs over four weeks of age may be carried out only under anaesthetic by a veterinarian or a qualified person.
- Neither tail docking nor tooth clipping must be carried out routinely but only if necessary. Where tooth clipping appears necessary, this must be carried out within seven days of birth.
- Piglets should not be weaned from the sow at less than three weeks of age, but it is possible to do it before, if necessary.

Weaners and rearing pigs:

- Pigs must be placed in groups as soon as possible after weaning.
- Mixing should be avoided as much as possible.

1.1.2. Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes

Articles:

- It is a general directive for all animals kept for farming purposes, including pigs.
- Member States shall guarantee that the owners or keepers ensure the welfare of animals and that those animals are not caused any unnecessary pain, suffering or injury.
- Member States shall ensure that inspections are carried out by the competent authority to check compliance of the provisions of this Directive.

Annex:

Staffing:

- Animals shall be cared for by a sufficient number of staff, who possess the appropriate ability, knowledge and professional competence.

Inspection:

- All animals shall be inspected at least once per day.
- Adequate lighting shall be available to enable a correct inspection.
- Any animal that appears to be ill or injured must be cared for appropriately, without delay. Where necessary, these animals shall be isolated in suitable accommodation.

Record keeping:

- The owner or keeper shall maintain a record of any medicinal treatment given and of the number of mortalities found at each inspection.
- These records shall be retained for a period of at least three years and shall be made available to the competent authority.

Freedom of movement:

- The freedom of movement of an animal must not be restricted in such a way as to cause it unnecessary suffering or injury.
- Where an animal is continuously or regularly tethered or confined, it must be given the space appropriate to its physiological and ethological needs.

Buildings and accommodation:

- Materials to be used for the construction of accommodation must not be harmful to the animals and must be capable of being thoroughly cleaned and disinfected.
- All environmental conditions must be kept within limits that are not harmful to the animals.
- Animals kept in building must have a correct period of lighting. If available natural light is insufficient, appropriate artificial lighting must be provided.

Animals not kept in buildings:

- They shall be given protection from adverse weather conditions, predators and risks to their health, when necessary and possible.

Automatic or mechanical equipment:

- All automated or mechanical equipment must be inspected at least once daily. Where defects are discovered, these must be rectified immediately, or appropriate steps must be taken to safeguard the health and well-being of the animals.
- An alarm system must be provided to give warning of breakdown when there is an artificial ventilation system. Its functioning must be tested regularly.

Feed, water and other substances:

- Animals must be fed a wholesome diet and at feeding intervals appropriate to their age and species and at a sufficient quantity to maintain them in good health and satisfy their nutritional needs. No animal shall be provided with food or liquid.
- All animals must have access to a suitable water supply.
- Feeding and watering equipment should avoid contamination of food and water and the harmful effects of competition.

Breeding procedures:

- Breeding procedures that produce suffering must not be practiced. Procedures that cause minimal or momentary suffering are acceptable.
- No animal shall be kept for farming purposes if it could be detrimental for its health or welfare.

1.1.3. Council Directive 2001/88/EC of 23 October 2001 amending Directive 91/630/EEC laying down minimum standards for the protection of pigs

Articles:

- As in the first directive, the floor area available for weaners and rearing pigs in groups is stipulated according to their weights, without any modification.
- The floor area available to each gilt and sow when kept in groups is established as at least 1.64 m^2 and 2.25 m^2, respectively.
- Flooring surfaces for pregnant gilts and sows should be at least 0.95 m^2 and 1.3 m^2 per animal, respectively, and it must be of continuous solid floor with drainage openings.
- The width of the openings of concrete slatted floors as well as the minimum slat width for pigs kept in groups is established regarding the category of the pig (piglets, weaners, rearing pigs or gilts/sows).
- Sow and gilt group housing are mandatory for the period starting from 4 weeks after the service to 1 week before the expected time of farrowing. The sides of the pen are stipulated depending on the number of animals.
- Sows and gilts shall have permanent access to manipulable material.
- Sows and gilts kept in groups must be fed using a system that ensures each individual can obtain sufficient food.
- To satisfy their hunger and given their need to chew, all dry pregnant sows and gilts must be given a sufficient quantity of bulky or high-fibre food as well as high-energy food.

- As in the preceding directive, more aggressive pigs or the ones suffering from the most aggression must be kept separate from the group but only temporarily.
- Every person engaging another person to attend to pigs should ensure that the keeper has received instructions and guidance on the relevant provisions of this directive.
- Training courses are available, in particular the ones regarding welfare aspects.
- The Commission shall submit to the Council a report, drawn up on the basis of an opinion from the Scientific Committee on Animal Health and Welfare, considering the development of techniques and systems to reduce the need for surgical castration. The Commission shall be assisted by the Standing Veterinary Committee and shall also be accompanied by the following:
    - appropriate legislative proposals about the different space allowances and floor types for weaners and rearing pigs to improve their welfare;
    - recommendations to reduce tail biting and tail docking;
    - further developments of group-housing systems for pregnant sows, considering pathological, zootechnical, physiological and ethological aspects, as well as the different climatic conditions; and
    - socioeconomic implications of the various systems.

1.1.4. Commission Directive 2001/93/EC of 9 November 2001 amending Directive 91/630/EEC laying down minimum standards for the protection of pigs

Articles:

- When pigs are kept in groups, appropriate management measures for their protection should be taken to ensure their welfare.
- Castration, tail docking, tooth clipping and tooth grinding cause acute and chronic pain to pigs, especially when carried out by incompetent persons. Rules should be established to ensure better practices.
- Piglets should not be weaned from the sow before four weeks of age, meaning one more week than in the first directive, but it is possible to do it before if necessary.

Annex:

Chapter 1 - General conditions:

In addition to the relevant provisions of the Annex to Directive 98/58/EC:

- Continuous noise levels as loud as 85 dBA shall be avoided.
- Lighting is mandatory at least eight hours per day (as in the first directive) and with an intensity of at least 40 lux.
- The accommodation for pigs must allow them to lie down, rest and stand up without difficulty; be clean; and see other pigs, as in the first directive. However,

in the week before the expected farrowing time and during farrowing, sows and gilts can be kept out of the sight of conspecifics.

- Pigs must have permanent access to a sufficient quantity of material to enable proper investigation and manipulation activities, such as straw, hay, wood, sawdust, mushroom compost, peat or a mixture of such, which does not compromise the health of the animals. The difference between the first directive and this one is the specification of the materials.
- Floors must be smooth but not slippery, as established by the first directive.
- All pigs must be fed once per day, and each pig must have access to the food at the same time as the others in the group, as in the first directive.
- All pigs over two weeks of age must have permanent access to a sufficient quantity of fresh water, as in the first directive, but this time it does not mention "other fluids", so it is mandatory to provide water to them.
- All routinely painful procedures shall be prohibited, with some exceptions (with almost all procedures being allowed in the end):
    - teeth clipping or grinding not later than the seventh day of life of the piglets;
    - boars' tusks reduction in length when necessary;
    - tail docking;
    - castration by means other than tearing of tissues;
    - nose ringing only when kept outdoors and in compliance with national legislation.
- Neither tail docking nor reduction of corner teeth must be carried out routinely but only where evidence exists of injuries to sows' teats or to other pigs' ears or tails. Before carrying out these procedures, other measures shall be taken to

prevent tail biting and other, changing inadequate environmental conditions or management systems.

  - Thus, the following exceptions make the banned procedures allowable.

- All these procedures shall only be carried out by a veterinarian or a person trained to do the procedures, and if practiced after the seventh day of life, a procedure shall only be performed under anaesthetic and additional prolonged analgesia by a veterinarian.
  - What changes here compared to the first directive is the additional prolonged analgesia and the week of life instead of four weeks.

Chapter 2 - Specific provisions for various categories of pigs:

Boars:

- Boar pens must allow the boars to turn around, hear, smell and see other pigs. The lying area must be dry and comfortable, with a minimum size of 6 m². A larger area must be provided when pens are used for service. Thus, these specifications are the same as in the first directive, except for the pens used for service, which now must be at least 10 m² and without any obstacles.

Sows and gilts:

- Measures shall be taken to minimise aggression in groups. No measures are specified.
- If necessary, pregnant sows and gilts must be treated against external and internal parasites, as in the first directive.
- If they are placed in farrowing crates, animals must be cleaned, as in the first directive.

- Suitable nesting material should be provided, unless it is not technically feasible for the slurry system used in the establishment.
    - So, it is not mandatory.
- A clean, adequately drained and comfortable lying area must be provided, as in the first directive.
- An area behind the sow or gilt must be available for the farrowing (natural or assisted), as in the first directive.
- Farrowing crates must have some means of protecting the piglets, as in the first directive.

Piglets:

- A sufficient floor to allow them to rest together at the same time, must be solid or covered with a mat, or be littered with straw or any other suitable material.
- Piglets must not be weaned from the sow at less than four weeks of age (instead of the three weeks indicated in the last directive), but it is possible to do it before, if necessary.

Weaners and rearing pigs:

- Mixing should be avoided as much as possible. If it is done, mixing should occur at as young an age as possible, preferably before or up to one week after weaning.
- When pigs are mixed, they shall be provided with adequate opportunities to escape and hide from other pigs.
- When signs of severe fighting appear, appropriate measures should be taken, such as providing plentiful straw to the animals, of if possible, other materials for investigation.

- Aggressors shall be kept separate from the group.
- The use of tranquillising medications to facilitate mixing shall be limited and only after consultation with a veterinarian.

1.1.5. Council directive 2008/120/EC of 18 December 2008 laying down minimum standards for the protection of pigs

Currently, this is the directive in effect.

- The floor area available for weaners and rearing pigs in groups is specified according to their weights, the same as in the first directive.
- The floor area available to each gilt and sow when kept in groups is the same as in the first directive.
- The use of tethers for sows and gilts shall be prohibited.
- As established in the first directive, sows and gilts shall be kept in groups during a period starting from four weeks after the service to one week before the expected time of farrowing. The dimensions are also specified regarding the number of sows.
- Sows and gilts should have permanent access to manipulable material.
    - This time, it is mandatory, with no exception.
- Sows and gilts kept in groups should be fed using a system which ensures that each individual can obtain sufficient food, as in the first directive.
- All pregnant sows and gilts, to satisfy their hunger and given their need to chew, must be given a sufficient quantity of bulky or high-fibre food as well as high-energy food.

- Aggressive pigs, pigs suffering from aggression, and sick and injured pigs may temporarily be kept in individual pens.
- Every person engaging another person to attend to pigs should ensure that the keeper has received instructions and guidance on the relevant provisions of this directive, as in the first directive.
- Training courses are available, in particular the ones regarding welfare aspects, as in the first directive.
- The Commission shall submit to the Council a report, drawn up on the basis of an opinion from the European Food Safety Authority. The report shall be drawn up considering the socioeconomic consequences, the sanitary consequences, the environmental effects and different climatic conditions. The report shall also consider the development of techniques and systems of pig production and meat processing that would be likely to reduce the need to resort to surgical castration, as in the first directive. If need be, the report shall be accompanied by appropriate legislative proposals on the effects of different space allowances and floor types applicable to the welfare of weaners and rearing pigs.
- To be imported into the Community, animals coming from a third country must be accompanied by a certificate of having received treatment at least equivalent to that stipulated in the present directive, as in the first directive.

Chapter 1 - General conditions:

- All the conditions stipulated are the same as in the last directive.

Chapter 2 - Specific provisions for various categories of pigs:

- All the conditions stipulated are the same as in the last directive.

1.2. Evolution, future and final considerations

This analysis of the law of protection of pigs allows us to make some conclusions and suggest final considerations.

First, we can see that the most important problems with animal welfare have been addressed in the law since the first directive, which seems to be the most effective one.

Second, the pig production industry constitutes a large lobby that does not allow some practices to really be eliminated or forbidden, even though scientific evidence proves some practices are not compatible with animal welfare principles. After all the scientific reports of experts and scientists and all the pressure produced by some animal protection organisations, some practices, such as the castration of piglets and other painful procedures, as well as early weaning and other procedures, have been addressed by the law. Nevertheless, they have not evolved much from the first to the last directive, frequently being addressed merely as recommendations or being allowed as an exception. These exceptions, in the end, result in the procedures occurring as normal procedures performed by most of the breeders, as they are legally permitted.

From our knowledge of the functioning of the pig production system, we can see that some of the articles or annexes are not being respected, such as the mandatory provision of manipulable material for all the pigs. This rule is known to all the community, the industry and the stakeholders, but the failure to provide manipulable material is not penalized by the inspectors (or not often enough), when there are inspections, as only a small percentage of farms are inspected. Therefore, research is encouraged by European agencies in these main difficult aspects to provide some solutions or methods and tools to be able to follow the law, thereby ensuring minimal standards of welfare, which is also a request of the consumers.

Importantly, we can see an evolution of the most important points regarding the protection of pigs, meaning that, even if slowly, each new directive is slightly stricter than the preceding one, with the most important points to ensure a minimum state of welfare is being considered, even if sometimes the directives are not efficient. We can also see some recommendations that change to obligations, although sometimes, they are not followed by the farmers.

Finally, our opinion is that, not only is the work of the scientific community essential to provide scientific evidence about the animal welfare problems in pig production systems but also proposals need to be made to solve these problems in a feasible and economical way. Only with this approach will the law continue to evolve, although it may evolve more slowly than scientific evidence, to ultimately ensure the minimum standards of the welfare of pigs. These minimum standards need to really ensure a better quality of life for these animals, not only for animal welfare but also for ethical reasons and for public health and food safety, as well as to satisfy the consumers and the society.